Green synthesis of zinc oxide nanoparticles from Pteris plant extract and it's antimicrobial activity

Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

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The dissertation work was carried out as a part of the academic requirement for the partial fulfillment of the degree of Master of Science in Biotechnology under the School of Health Science and Translational Research, Sister Nivedita University, Kolkata.

The research project entitled *"Green synthesis of zinc oxide nanoparticles from Pteris plant extract and it's antimicrobial activity"* was conducted during the period January 2025 to June 2025, and the student has shown sincere dedication and scientific aptitude throughout the course of the work.

I wish the student every success in future academic and professional endeavors.

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Abbreviations:

- **ZnO-NPs** – Zinc oxide Nanoparticles
- **FTIR** – Fourier Transform Infrared Spectroscopy
- **DLS** – Dynamic Light Scattering
- **XRD** – X-ray Diffraction
- **UV-Vis** – Ultraviolet-Visible Spectroscopy

ABSTRACT:

Zinc plays a vital role in both biological systems and industrial applications, and its oxide form—zinc oxide (**ZnO**)—has become especially important in nanotechnology. ZnO nanoparticles (**ZnO-NPs**) are widely used today due to their impressive antimicrobial, antioxidant, and UV-protective properties. However, the conventional ways of producing these nanoparticles often rely on harmful chemicals, high temperatures, and complex lab setups, which pose environmental and health risks.

In this study, we explored a safer, greener method by using the ***Pteris vittata*** plant to synthesize **ZnO-NPs**. This fern is not only rich in natural compounds like **flavonoids**, **terpenoids**, and **phenolics** but is also known for its ability to absorb heavy metals from the environment. By using an aqueous extract of ***Pteris vittata***, we successfully produced zinc oxide nanoparticles under mild, eco-friendly conditions—no toxic chemicals required.

The nanoparticles were then characterized using various techniques such as **UV-Visible spectroscopy, Dynamic Light Scattering (DLS), X-Ray Diffraction (XRD), and FTIR**. These tests confirmed that the nanoparticles were well-formed, relatively stable, and sized between **20** to **150** nanometers.

To understand their practical value, we tested the antimicrobial activity of the **ZnO-NPs** against common bacteria like ***E. coli***, ***Staphylococcus aureus***, and ***Bacillus subtilis***. The results were promising: the nanoparticles effectively inhibited bacterial growth, especially at higher concentrations, and worked by damaging the bacterial membranes and creating oxidative stress.

Overall, this study shows that Pteris vittata is not just a plant, but a natural bio-factory capable of producing useful, biocompatible nanoparticles. This green approach to nanomaterial synthesis holds real potential for safe and sustainable applications in medicine, agriculture, and environmental science.

Keywords: Zinc oxide nanoparticle; Green synthesis; UV- visible spectroscopy; DLS; XRD; FTIR; Antimicrobial activity

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Chapter: 1

Introduction:

Nanotechnology, or the manipulation of matter at the atomic and molecular level, has revolutionized several scientific fields of study (1), most notably environmental studies, materials engineering, and medicine (2). Among the various **nanomaterials** being studied, **zinc oxide nanoparticles (ZnO-NPs)** have attracted significant attention due to their unique physicochemical properties (3). The most notable among these are their strong **antimicrobial** activity against a wide-ranging of microbes, high surface area-to-volume ratio, excellent photocatalytic performance, UV-blocking ability (4), and robust chemical stability**. ZnO-NPs** are currently used in a wide range of applications, including sunscreens, cosmetics, antibacterial coatings, food -packaging, water waste treatment (5), and as additives in paints and rubber.(6)

Although zinc-based materials have been used for centuries, the systematic production and documentation of zinc oxide nanoparticles (ZnO- NPs) began much later (7), primarily emerging with the advancement of nanotechnology in the late 20th and early 21st centuries.(8) While ancient societies were not explicitly aware of nanoparticles, they did utilize zinc compounds for various purposes, such as in ointments and medicinal preparations (9). The **nanoscale properties** of zinc oxide, however, were not fully understood or intentionally applied in historical artifacts like silver or gold nanoparticles (10). The purposeful synthesis and use of ZnO nanoparticles for their unique optical, antibacterial, and catalytic properties is a relatively modern development, driven by advancements in materials science and nanotechnology.

Milestone	Contributor	Year	Significance
Colloidal metal - nanoparticles (Ag)	**Michael Faraday**	**1857**	**Initiated the process for nanoparticle research**
Chemical synthesis of silver nanoparticles	**Creighton et al.**	**1979**	**Employing reducing agents, the first modern-day synthesis**
Characterization and synthesis of ZnO-NPs	**Wang et al. (commonly cited)**	**2004**	**Demonstrated controlled synthesis and size-dependent properties of ZnO nanoparticles**

Table 1.1: Key Milestones in the Development of Nanoparticle Synthesis and Characterization.

Nanoparticles (NPs) are very small materials with at least one-dimension in the nanometer range, typically between **1** and **100 nanometers (nm)**. (11) At this scale range, materials exhibit their unique physicochemical abilities **due to their high surface area-to-volume ratio**, **increased surface energy, and quantum confinement effects**.(12) These characteristics result in distinct optical, electrical, magnetic, catalytic, and biological properties compared to their bulk counterparts. Nanoparticles can be composed of various materials, including metals (like **silver**, **gold**, and **zinc**), metal oxides (such as **TiO_2** and **ZnO**),(13) polymers, lipids, and ceramics. Among these, zinc oxide nanoparticles also (ZnO- NPs) have gained their significant attention due to their remarkable photocatalytic activity, UV-blocking capability, and strong antimicrobial and anti-inflammatory properties.(14) These features make ZnO-NPs highly valuable in diverse fields such as environmental remediation, agriculture, food preservation, cosmetics, sunscreen formulations, and biomedical applications.(15)

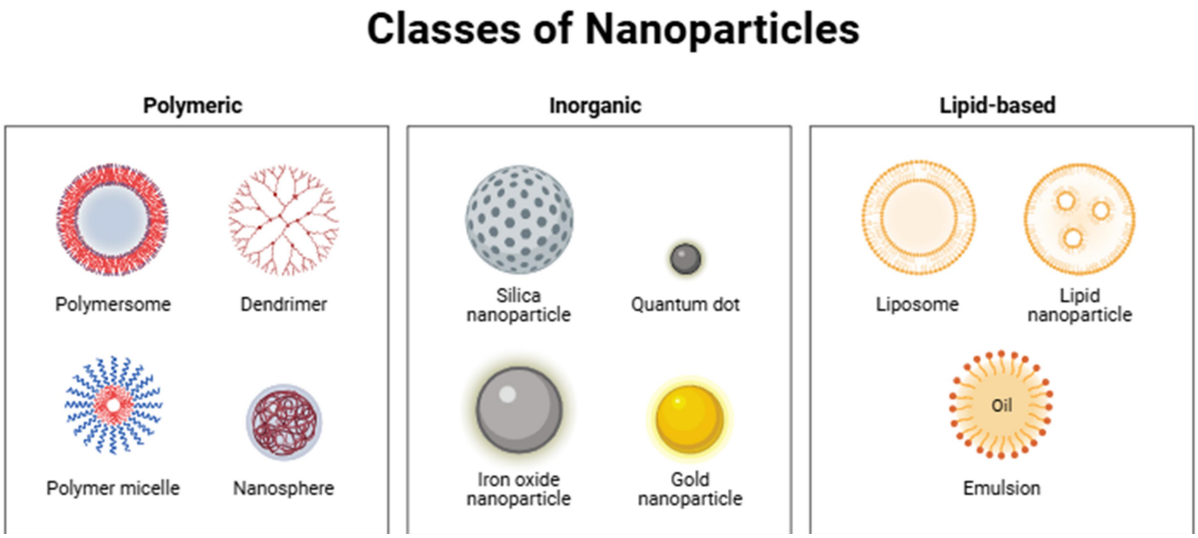

Fig: 1.1 Classes of different nano particles.

Nanoparticles organized them into different categories:

Type of Nanoparticle	Composition	Essential Characteristics	Applications	Nanotechnology Relevance
Metal Nanoparticles	Silver (Ag), Gold (Au), Copper (Cu)	Antimicrobial, Optical (SPR), Conductive	Antibacterial agents, Imaging, Sensors	Nano-optics, Biomedical nano-sensors
Metal Oxide Nanoparticles	ZnO, TiO_2, Fe_3O_4	Photocatalytic, Magnetic, UV-absorbing	Sunscreens, MRI contrast, Wastewater treatment	Environmental nanotech, Magnetic nanotech
Carbon-Based Nanoparticles	CNTs, Fullerenes, Graphene	High strength, Conductivity, Lightweight	Electronics, Drug delivery, Composite materials	Nanoelectronics, Structural nanomaterials
Polymeric Nanoparticles	PLGA, PEG-based polymers	Biodegradable, Biocompatible, Controlled release	Drug delivery, Vaccine carriers	Pharmaceutical nanotech
Lipid-Based Nanoparticles	SLNs, Liposomes, NLCs	Biocompatibility, Targeted release	Nutraceuticals, Cosmetics, Drug delivery	Nano-pharmaceutical formulations
Quantum Dots	CdSe, PbS (semiconductors)	Size-tunable fluorescence, Optical stability	Bioimaging, Solar cells, Diagnostics	Nano-optics, Nanoimaging
Dendrimers	Synthetic branched polymers	High functionality, Nanoscale size	Targeted drug/gene delivery, Imaging	Biomedical nanotechnology
Ceramic Nanoparticles	Silica, Alumina	High stability, Porosity	Bone regeneration, Catalysis	Biomedical and catalytic nanotech
Magnetic Nanoparticles	Fe_3O_4, $CoFe_2O_4$	Magnetic responsiveness, Biocompatibility	MRI, Targeted therapy, Data storage	Magnetic nanotechnology, Medical nanodevices
High-Entropy Alloy Nanoparticle	Multi-metallic systems	Thermal stability, Catalytic synergy	Catalysis, Energy storage	Advanced materials nanotechnology

Table no 1.2: Overview of Nanoparticle Types and Their Technological Relevance.

Conventional synthesis methods for **ZnO** nanoparticles, including sol-gel processing, hydrothermal techniques, precipitation methods, and physical approaches like vapor deposition, are effective in the producing all nanoparticles with controlled size and morphology. However,

these methods were often involve into the use of hazardous precursors, high temperatures or pressures, and complex laboratory setups. These limitations not only elevate production costs but also pose environmental and health risks due to the generation of toxic by-products and non-biodegradable residues.

A wide spectrum of biological organisms has emerged as key agents in the eco-friendly synthesis of nanoparticles, playing an important role in the advancement of **green nanotechnology** (16). Among these, six primary groups—**plants, algae, bacteria, fungi, virus, and yeast**—are widely used due to their natural ability to reduce metal ions into stable nanoparticles without the need for toxic chemicals. Each of these organisms contributes unique bioactive molecules, such as proteins, polysaccharides, enzymes, phenolic compounds, flavonoids, and alkaloids,(17) which actively participate in the **reduction**, **stabilization**, and **capping** of nanoparticles. For instance, **plant extracts** are rich in **secondary metabolites** that not only speed up the reaction but also allow for **size** and **shape** control of the nanoparticles. **Bacteria** and **fungi** are also highly efficient producers due to their fast growth rates and the ease with which they can be genetically manipulated or cultured under laboratory conditions (18). **Viruses**, particularly virus-like particles (**VLPs**), it's engineered for viral nanoparticles, have gained significant attention for their ability to self-assemble into uniform nanostructures and are increasingly explored for the production of nanoparticles with distinct physicochemical properties.(19) **Algae** and **yeast**, although less frequently used, offer additional benefits such as high metal tolerance, scalability, and the ability to operate in varied environmental conditions. The involvement of these biological organisms enables a cleaner, more sustainable approach compared to traditional physical and chemical synthesis methods,(20) which often involve harsh conditions, toxic reagents, and high energy input. The nanoparticles synthesized using biological agents are generally biocompatible, it's making them highly suitable for use in biomedical applications including **targeted drug delivery**, **wound healing**, **antimicrobial treatments**, **imaging**, and **diagnostics**. Ongoing research continues to explore and optimize the roles of these organisms, aiming to improve yield,(21) control particle properties more precisely, and expand their application scope across industries beyond medicine, such as a**griculture, food preservation, and environmental remediation.**

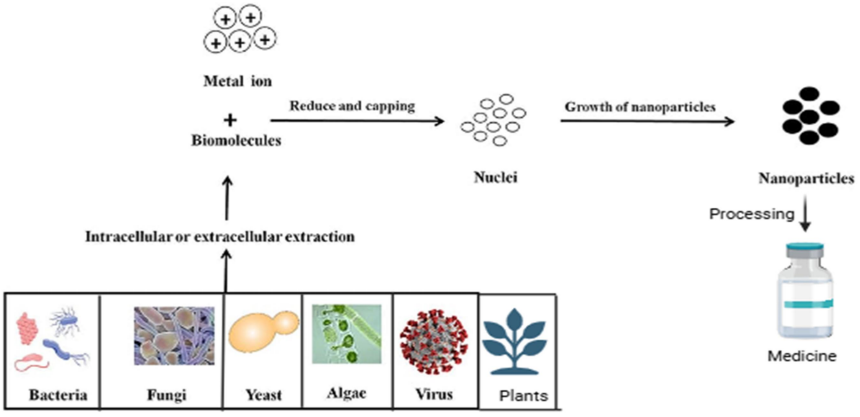

Fig 1.2: Nanoparticle synthesis by 6 primary groups of organism (plants, algae, bacteria, fungi, virus, and yeast)

Green synthesis has gained popularity as an effective and sustainable solution to the limitations of conventional ZnO nanoparticle production.(22) This eco-friendly approach utilizes the **reducing** and **stabilizing capabilities** (23) of biological entities such as bacteria, fungi, algae, and plant extracts to synthesize ZnO nanoparticles under mild conditions.(24) Among these biological sources, plant-mediated synthesis stands out due to its **simplicity**, **cost-effectiveness**, **scalability**, and the **abundance** of **phytochemicals** in plants that serve as **natural reducing** and **capping agents**.(25) This method is not only removed the need for toxic chemicals but also aligns with environmentally conscious manufacturing practices.(26)

Plant Name	Scientific Name	Types of Nanoparticles Synthesized	Key Bioactive Compounds
Neem	*Azadirachta indica*	Silver (Ag), Gold (Au), Zinc (Zn) etc.	Flavonoids, terpenoids, tannins and azadirachtin
Tulsi (Holy Basil)	*Ocimum sanctum*	Silver (Ag), Gold (Au)	Eugenol, flavonoids, tannins, alkaloids
Green Tea	*Camellia sinensis*	Silver (Ag), Gold (Au)	Polyphenols (catechins), flavonoids

Aloe vera	*Aloe barbadensis miller*	Silver (Ag), Iron Oxide (Fe_2O_3)	Anthraquinones, flavonoids, carbohydrates, saponins
Moringa	*Moringa oleifera*	Silver (Ag)	Ascorbic acid, flavonoids, phenolics
Eucalyptus	*Eucalyptus spp.*	Silver (Ag), Iron Oxide (Fe_2O_3)	Tannins, flavonoids, terpenoids
Turmeric	*Curcuma longa*	Gold (Au)	Curcumin, flavonoids, phenolics
Ginger	*Zingiber officinale*	Silver (Ag), Zinc (Zn)	Gingerol, shogaol, flavonoids
Pteris	*Pteris vittata*	Zinc (Zn), Silver (Ag)	Flavonoids, phenolic acids, terpenoids

Table no 1.3: The Natural Green Alchemists: The Synthesis of Nanoparticles by Plants:

Numerous plants, including **neem**, **tulsi**, **green tea**, **aloe vera**, and **turmeric**, (27) can naturally produce nanoparticles. Their extracts contain bioactive compounds that facilitate the conversion of metal salts into nanoparticles of zinc, gold, and silver. This method is widely recognized as "**plant-based**" or "**green" synthesis**. (28)

Pteris vittata, commonly known as the **Chinese brake fern**, is one such plant that has shown significant potential in this field.(29) Belonging to the ***Pteridaceae*** family, ***Pteris vittata*** is a well-known **hyperaccumulator** of heavy metals like arsenic and is often used in **phytoremediation**. Recent studies have also highlighted its ability to act as a bio-factory for the green synthesis of zinc oxide (ZnO) nanoparticles. (30) This ability is attributed to its rich phytochemical composition, which includes flavonoids, phenolic acids, terpenoids, and other secondary metabolites known for their strong reducing and stabilizing capabilities.(31)

The use of ***Pteris vittata*** in nanoparticle synthesis offers multiple advantages, including eco-friendliness, cost-effectiveness, and scalability. ZnO nanoparticles derived from ***Pteris* extracts** have demonstrated promising **antimicrobial** and **antioxidant activities**, making them suitable for various biomedical applications.(32) These include antimicrobial coatings, wound-healing materials, drug delivery systems, and environmental sensors. The renewable nature, fast growth, and widespread availability of ***Pteris vittata*** further enhance its appeal as a sustainable resource for **nanoparticle production**. (33)

Chapter: 2

Review of Literature

2.1. Nanoparticle synthesis approaches:

2.1.1. **Overview of Nanoparticle Synthesis Strategies**: In the past few years, diverse techniques have been explored for synthesizing nanoparticles, including physical, chemical, and biological methods. (34) These techniques broadly fall under two main strategies: the **top-down approach**, which is physical in nature, and **the bottom-up approach**, it's includes the both chemical and biological methods. The top-down approach includes the breaking down all bulk materials into **nanoscale structures** through techniques like thermal decomposition, irradiation, diffusion, and arc discharge. (35) In contrast, **the bottom-up approach** builds nanoparticles molecules by molecules through techniques such as chemical reduction, electrochemical synthesis, polyol process, seeded growth, and biosynthesis .(36)

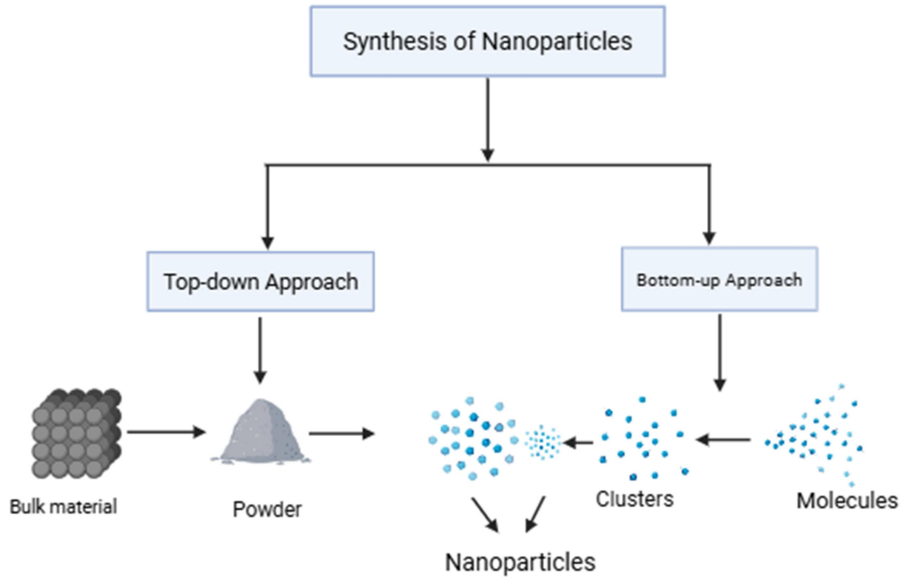

Fig 2.1: Difference between Top-down and Bottom down approach

2.1.2. **Top-Down vs. Bottom-Up Approaches:** They are both of chemical and physical synthesis methods are well-established, they often come with significant

drawbacks.(37) These include the use of toxic reducing agents (e.g., sodium borohydride), hazardous solvents, exposure to UV and gamma radiation, and high energy requirements—all of which pose environmental and health risks. Due to these limitations, researchers are increasingly interested in developing eco-friendly alternatives for nanoparticle production. (38)

2.1.3. **Limitations of Conventional Methods** In between two synthesis biological synthesis methods have gained notable attention. These are involves into the use of biological systems—such as microorganisms and plants—to synthesize nanoparticles. This approach, often categorized under **nanobiotechnology**, aligns with natural biochemical processes, offering a cleaner, safer, and more sustainable route to nanoparticle production (39). Nanobiotechnology essentially utilizes biological molecules and systems for designing and producing **nanoscale materials**, drawing inspiration from natural nano-machinery observed in living cells.

2.1.4. **Emergence of Biological Synthesis (Nanobiotechnology)**: Nature itself has provided compelling examples of biosynthesis. For example, **magnetotactic bacteria** are produce magnetite particles (40;41), diatoms form silica-based structures, and S-layer bacteria generate gypsum and calcium deposits. These act of nature have inspired the use of microorganisms—including bacteria, actinomycetes, fungi, and algae—for nanoparticle synthesis (42). Additionally, many variety of plants extracts from leaves, flowers, bark, seeds, and peels all have also been used for green synthesis of nanoparticles (43).

2.1.5. **Microbial and Plant-Based Nanoparticle Synthesis** Biological synthesis can occur via two pathways: intracellular and extracellular. In intracellular synthesis, nanoparticles form within the cells (44), while in extracellular synthesis, secreted enzymes and biomolecules facilitate nanoparticle formation outside the cell (45).

2.1.6. **Role of *Pteris* Plant in Nanoparticle Synthesis**: In between various biological agents, the **Pteris** plants has appeared as a promising candidate for nanoparticle synthesis. This plant is known for its remarkable ability to uptake and accumulate heavy metals from the environment, a trait that plays a vital role in nanoparticle formation.(46) The green synthesis approach using **Pteris** offers several

advantages, including low cost, environmental friendliness, ease of handling, and potential for large-scale application. These properties are make Pteris as a valuable and useful botanical resource for sustainable nanoparticle production.(47) Recent studies have highlighted the efficiency of ***Pteris vittata*** and related species in synthesizing metal nanoparticles, reinforcing their potential in nanobiotechnology and environmental remediation (48). Thus, the use of **Pteris** plants offers a **natural, eco-conscious alternative to traditional chemical and physical synthesis** methods.

2.2. Chemical Synthesis vs. Biosynthesis of Nanoparticles: Nanoparticle synthesis has traditionally relied on **chemical methods**, which often involve harsh reaction conditions, toxic reducing agents, and the generation of hazardous byproducts. These approaches, while offering precise control over particle size and morphology, raise environmental and biocompatibility concerns (49). In contrast, **biosynthesis**, which utilizes biological entities such as **plants, bacteria, fungi, and viruses**, has emerged as a **greener and more sustainable alternative**. This method not only reduces the use of harmful chemicals but also leverages the natural reducing and capping abilities of biological systems to produce the nanoparticles with better stability and functional properties (50). Moreover, biosynthesized nanoparticles often exhibit improved biocompatibility which making them particularly attention for biomedical applications.

2.3. **Used of plants in ZnO-NPs Synthesis:** A growing body of research highlights the successful green synthesis of the zinc oxide nanoparticles (ZnO-NPs) by using various plant species. According to a comprehensive study by Sharma et al. (51), more than 80 plant species across diverse families have been utilized for **ZnO-NPs biosynthesis**.(52) These include families such as **Asteraceae**, **Lamiaceae**, **Zingiberaceae**, and **Meliaceae**. Different parts of the plant like leaves, roots, bark, flowers, fruits, and peels—contain distinct phytochemicals that serve as reducing and capping agents during nanoparticle formation.(53)

The study emphasized the efficient synthesis of ZnO-NPs using extracts from:

- **Aloe vera** (***Asphodelaceae***):It's rich in **saponins** and **flavonoids** that enhance nanoparticle stability

- ***Azadirachta indica*** (Meliaceae): It's Provides **terpenoids** and polyphenols aiding in nanoparticle reduction
- ***Camellia sinensis*** (Theaceae): Contains **catechins** and **flavonoids** with strong antioxidant properties
- ***Curcuma longa*** (Zingiberaceae): **Curcumin** in turmeric plays a useful role in the nanoparticle stabilization
- ***Ocimum sanctum*** (Lamiaceae): It's known for its high content of **eugenol, flavonoids,** and **tannins**
- ***Zingiber officinale*** (Zingiberaceae): Synthesized **ZnO-NPs** with **gingerol** and **shogaol** acting as **bioreducers**
- ***Moringa oleifera*** (Moringaceae): Ascorbic acid and **phenolics** in leaves facilitate nanoparticle synthesis
- **Punica granatum** (Lythraceae): Peel extracts; it's contain **tannins** and **phenolic acids** beneficial for ZnO-NPs formation
- ***Tridax procumbens*** (Asteraceae): Leaf extract serves as a potent bio-reducing medium
- ***Pteris vittata*** (Pteridaceae): It's a metal-hyperaccumulating fern which was recently explored for ZnO-NPs biosynthesis due to its rich flavonoid and phenolic content

These examples have the immense potentials of the plant biodiversity in the eco-friendly synthesis of zinc oxide nanoparticles. As research expands, more plant species are being investigated, not only for their synthesis capability but also for the enhanced antimicrobial, antioxidant, and catalytic activities imparted by the nanoparticles they help produce.

2.4. Overview of Green Synthesis of ZnO Nanoparticles Using Pteris Extract: The green synthesis of zinc oxide nanoparticles (ZnO-NPs) using **Pteris plant** extract represents a promising advancement in **eco-friendly nanotechnology**. This method utilizes the rich phytochemical profile of the **Pteris** genus—such as **flavonoids**,

terpenoids, and **phenolic** compounds—they are act like as a **reducing** as well as **stabilizing** agents in the nanoparticle synthesis process. The approach removes the need for toxic chemicals commonly used in conventional methods, thereby reducing environmental risks and making the process more **biocompatible**. Studies have shown that Pteris-mediated ZnO-NPs exhibit notable antimicrobial and antioxidant activities, attributed to the synergistic effect of the nanoparticle surface chemistry and bioactive **phytoconstituents** derived from the extract. These features make them highly suitable for biomedical and environmental applications.(54,55).

2.5. Phytochemical Composition of Pteris Extract: The Pteris genus, a group of ferns known for their **hyperaccumulating** ability and medicinal use (56), contains a variety of bioactive compounds that contribute to the stabilization and production of Zinc oxide nanoparticles:

- **Flavonoids:** These are potent antioxidants with reducing properties that facilitate the transformation of zinc ions (Zn^{2+}) into zinc oxide nanoparticles.(57) They also play a key role in stabilizing the nanoparticles and controlling their morphology.(58)
- **Phenolic compounds**: Known for their strong electron-donating capacity, phenolics contribute to the reduction of zinc salts and enhance the overall stability and bioactivity of the ZnO-NPs.(59)
- **Terpenoids and saponins**: These act as natural surfactants, helping to regulate both the size and prevent aggregation of nanoparticles, thereby maintaining their functional integrity.

The use of **Pteris extract** in ZnO-NPs synthesis not only promotes an eco-friendly route of nanoparticle production but also imparts biofunctional properties that can be harnessed in biomedical, agricultural, and environmental applications. This plant-mediated method offers **simplicity**, **cost-effectiveness**, and **reduced toxicity**—making it a viable alternative to conventional chemical synthesis techniques. (60)

2.6. Phytochemical Profiling of *Pteris* Species for Green Synthesis of Zinc Oxide Nanoparticles: The effectiveness of Pteris species in the green synthesis of zinc oxide nanoparticles (ZnO-NPs) is attributed to their diverse and rich phytochemical composition. (61)These naturally occurring compounds not only enable the eco-

friendly reduction of metal ions but it's also influence the characteristics of the manufactured nanoparticles.(62)

Pteris plant extract contains many bioactive constituents, which are including:

- **Flavonoids and phenolic acids** – It's known for their strong antioxidant and electron-donating capabilities
- **Tannins** – which provide reducing and stabilizing action
- **Terpenoids and glycosides** – It's important for the shape and size control during nanoparticle formation
- **Saponins** – function as natural surfactants and stabilizing agents
- **Alkaloids and steroids** – which may aid in particle nucleation and antimicrobial properties
- **Polysaccharides and proteins** – acting as biocompatible capping agents to prevent aggregation
- **Essential minerals** – like zn^{2+}, calcium, and iron etc.

In the green synthesis process, flavonoids and phenolic compounds donate electrons to reduce Zn^{2+} ions into ZnO nanoparticles,(63) initiating nucleation. Once nucleation begins, saponins, proteins, and polysaccharides act as a natural capping and stabilizing reagent, forming a biocompatible shell around the nanoparticles. (64) This prevents agglomeration and enhances nanoparticle dispersion in aqueous media.

Additionally, terpenoids and glycosides are useful in controlling the shape and size of the ZnO-NPs, ensuring a **uniform morphology**, which is crucial for biomedical and industrial applications.(65) These phytochemicals collectively make Pteris extract a highly efficient and eco-friendly medium for the synthesis of ZnO nanoparticles.(66)

Beyond synthesis, these compounds are also help to increase the biological activity of the resulting nanoparticles.(67) The **antimicrobial, antioxidant,** and **wound-healing properties** of ZnO-NPs synthesized by using Pteris extract are notably improved, making them suitable for biomedical, environmental, and cosmetic applications. (68)

2.7. Mechanism of Synthesis: The synthesis of the zinc oxide nanoparticles (ZnO-NPs) by using the Pteris plant extract (69), the process involves the following key steps:

- **Reduction of Zinc Ions: Zinc ions (Zn^{2+})** are reduced and transformed into zinc oxide nanoparticles (ZnO-NPs) by the electron-donating action of phytochemicals present in Pteris extract, (70) such as flavonoids and phenolic compounds. (71) This initiates the nucleation process essential for nanoparticle formation.

  Zn^{2+}+2OH- → heat → ZnO+ H_2O

- **Stabilization and Capping of ZnO-NPs**: Bioactive compounds like saponins, proteins, and polysaccharides in the Pteris extract act as natural stabilizing and capping agents. These substances form a **protective**,(72) **biocompatible shell** around the nanoparticles, which prevents agglomeration and enhances stability.(73)
- **Shape and Size Control:** Terpenoids and glycosides in the Pteris extract further assist for regulating the morphology (shape and size) of the synthesized ZnO-NPs, resulting in nanoparticles with desirable properties for it's biomedical and antimicrobial applications.(74)

2.8. Advantages of Pteris Plant for Green Synthesis of ZnO Nanoparticles:

a) **Eco-Friendly and Sustainable Technique:** The green synthesis of ZnO nanoparticles using Pteris extract avoids the use of hazardous chemicals and energy-intensive methods.(75) This environmentally benign process supports sustainable nanotechnology and stands with the several **UN Sustainable Development Goals** (SDGs).

b) **Economic Sustainability:** Pteris plants are naturally abundant, easy to cultivate, and require minimal processing. This makes them a **cost-effective** and scalable option for large-scale ZnO-NPs production, especially in **resource-limited** settings.(76)

c) **Biocompatibility:** ZnO nanoparticles synthesized using Pteris extract are biocompatible and free from toxic residues. This makes them ideal for biomedical and pharmaceutical applications, including **antibacterial formulations, drug delivery,** and **tissue engineering**.(77)

d) **Improved Stability:** The natural proteins, polysaccharides, and saponins in Pteris extract act as capping agents that prevent agglomeration. This gives impact on high colloidal stability and increases the **shelf life** of the all nanoparticles.(78)

e) **Controllable Physical Properties:** Parameters like extract concentration, pH, reaction time, and temperature can be varied to effectively control the size, shape, and surface morphology of ZnO-NPs. The diverse phytochemical composition of Pteris aids in tunable synthesis for specific functional applications.(79)

f) **Antioxidant and Antimicrobial Properties:** Natural flavonoids and phenolics in Pteris provide **antioxidant protection** during nanoparticle synthesis. These properties not only stabilize ZnO-NPs but also enhance their **antimicrobial efficacy**, making them valuable for health care and environmental applications.(80)

2.9. Role of Pteris Plant in the Synthesis of Nanoparticles: Among the many plant sources explored for green synthesis, (81) the Pteris plant (a fern belonging to the **Pteridaceae** family) has surfaced as a promising candidate for it's eco-friendly nanoparticles production. The extract of the Pteris leaves is a rich in bio-active phytochemicals such as **flavonoids**, phenolic-compounds, **saponins, glycosides, terpenoids**, **alkaloids**, and **poly-saccharides**.(82) These compounds serve both roles in the synthesis of ZnO nanoparticles:

- As **reducing agents**, flavonoids and phenolics donate electrons to convert **Zn^{2+}** ions into ZnO nanoparticles.(83)
- As **stabilizing and capping agents**, polysaccharides, proteins, and saponins coat the nanoparticles, preventing agglomeration and ensuring uniform shape and dispersion.(84)

The use of Pteris in nanoparticle synthesis offers multiple advantages:

- **Non-toxic** and **biodegradable** synthesis method
- **Dual functionality**,behave as reducing as well as stabilizing reagent.
- **Sufficient** and **renewable natural resource**, provide them medicinal and ecological significance
- **Biocompatibility**, it's making suitable for biomedical and environmental applications.

A study by demonstrated (85) the efficient formation of ZnO nanoparticles using ***Pteris vittata*** extract under mild conditions, producing uniformly dispersed particles with potent antimicrobial

activity. This confirms the viability and effectiveness of Pteris-based **green synthesis** in modern nanotechnology.(86)

2.10. **Applications of Pteris-Mediated Zinc Oxide Nanoparticles:** Zinc oxide nanoparticles synthesized via Pteris plant extract exhibit a wide range of applications due to their excellent **physicochemical stability**, **biocompatibility**, and **strong biological activity**, especially their antibacterial, antifungal, antioxidant, and UV-protective properties. These characteristics makes them Pteris-mediated ZnO-NPs highly valuable in **medicine, environmental protection**, and **agriculture.**

2.10.1. **Biomedical Applications:** Pteris-based ZnO-NPs are especially promising into the **pharmaceutical** and **healthcare industries** due to their better biological properties given by the phytochemicals involved in their **green synthesis** process.

a) Wound Healing and Antiseptic Treatments: ZnO-NPs synthesized from Pteris extract possess potent **antimicrobial** and **anti-inflammatory** properties, making them ideal for topical applications in burns, ulcers, and wounds. The flavonoids and phenolics from Pteris help in tissue repair and healing.

- Promote **fibroblast proliferation** and **collagen** formation
- Support **re-epithelialization** and **angiogenesis**
- **Inhibit microbial colonization** and reduce **wound infections**

These characteristics make them effective ingredients in ointments, hydrogels, sprays, and wound dressings.

b) Antibacterial and Antifungal Therapy: ZnO-NPs have demonstrated effectiveness against many types of antibiotic-resistant bacterial strains, such as ***Staphylococcus aureus***, and fungi like ***Candida albicans***. Pteris-derived ZnO-NPs can:

- Penetrate microbial membranes and cause **oxidative damage**
- **Interfere with DNA replication** and **protein synthesis**
- Disrupt biofilms, crucial in **chronic infections**

This makes them suitable for coatings on surgical instruments, implants, and catheters.

c) **Targeted Drug Delivery:** Thanks to their nano-size, high surface-to-volume ratio, and surface functional groups, Pteris-mediated ZnO-NPs can be tailored for drug loading and release, including:
   - Anticancer drugs (e.g., doxorubicin)
   - Anti-inflammatory agents
   - Antibiotics (e.g., ciprofloxacin)

These features improve the targeted delivery, enhancing treatment effectiveness while reducing side effects.

d) **Cancer Therapy:** Preliminary in vitro studies suggest that Pteris-synthesized ZnO-NPs can induce oxidative stress and apoptosis in cancer cells such as **MCF-7 (breast cancer)** and **HeLa (cervical cancer).**
   - Exhibit **selective cytotoxicity** toward cancer cells
   - Minimize damage to healthy tissues
   - Offer potential for **photodynamic** and **chemotherapeutic** applications (87)

2.10.2. Environmental Applications: Pteris-mediated ZnO-NPs are effective agents in **environmental remediation**, particularly in **wastewater treatment** and **antimicrobial surface applications.**

a) **Water Disinfection and Filtration:** ZnO-NPs can be embedded in filtration membranes or coating materials to disinfect drinking water by eliminating pathogens.
   - Kill bacteria, viruses, and fungi
   - Prevent biofilm formation in filters
   - Release minimal toxic byproducts, making them safer than conventional disinfectants

b) **Antimicrobial Surface Coatings:** These ZnO-NPs can be used on hospital surfaces, masks, gloves, and high-touch surfaces (like door handles and elevator buttons) to reduce **pathogen transmission**.

- Provide long-lasting **antimicrobial protection.**
- Reduce environmental toxicity as compared to the chemically synthesized ZnO-NPs (88)

2.10.3. **Agricultural Applications:** ZnONPs derived from Pteris extracts are an eco-friendly alternative to synthetic agrochemicals, offering antibacterial, antifungal, and plant-growth-promoting properties.

a) Control of Plant Pathogens: ZnO-NPs can control diseases caused by plant pathogens such as:

- ***Ralstonia solanacearum*** (bacterial wilt)
- ***Alternaria spp***. (leaf spot)
- ***Fusarium oxysporum*** (root rot)

They act by disrupting cell walls, metabolic processes, and genetic material of phytopathogens, serving as a biopesticide.

b) Seed Germination and Plant Growth: Low concentrations of ZnO-NPs improve:

- Seed germination rates
- Root elongation and shoot development
- Nutrient absorption and plant biomass

However, the effect is dose-dependent; high concentrations may be phytotoxic.

c) Food Packaging and Preservation: The antimicrobial properties of ZnO-NPs make them ideal for active food packaging. They can be incorporated into:

- Biodegradable films (e.g., chitosan or starch-based)
- Edible coatings for fruits and vegetables
- Antimicrobial wrapping materials

These applications help prevent spoilage by organisms like ***Listeria monocytogenes*** and ***Salmonella enterica***, thus enhancing shelf life and reducing food waste.(89)

2.11. Pteris-Mediated Formation of Zinc Oxide Nanoparticles (ZnO-NPs): The physicochemical properties of zinc oxide nanoparticles (ZnO-NPs)—such as their

morphology, particle size, surface area, crystallinity, and **dispersion**—significantly influence their optical, antibacterial, and catalytic activities. When synthesized using Pteris extracts, ZnO NPs typically exhibit the following features:

- They often possess **hexagonal wurtzite crystal** structures and exhibit spherical, rod-like, or irregular morphologies, depending on the phytochemical composition of the extract and synthesis parameters.
- Their particle sizes generally range from 20 to 100 nanometers, though finer control during synthesis can produce smaller sizes.
- The nanoparticles may show good dispersion or slight agglomeration, influenced by the concentration of biomolecules (e.g., polyphenols, flavonoids, proteins) in **Pteris extract**, which act as both reducing and stabilizing agents.

To confirm the synthesis and analyze the characteristics of Pteris-mediated ZnO NPs, several analytical techniques are employed:

- **UV-Visible Spectroscopy:** Shows a characteristic absorption peak in the range of **350–380 nm**, attributed to the **excitonic transitions** of ZnO and its **surface plasmon resonance-like behavior**.
- **Fourier-Transform Infrared Spectroscopy (FTIR):** Identifies the **functional groups (e.g., –OH, –COOH, –NH_2)** from the plant extract involved in the **reduction of Zn^{2+} ions** and the **stabilization of the ZnO NPs.**
- **Transmission Electron Microscopy (TEM)** and **Scanning Electron Microscopy (SEM):** Reveal the size, shape, and morphology of the nanoparticles.
- **X-ray Diffraction (XRD):** Confirms the **crystalline nature** and phase purity of the ZnO nanoparticles, typically showing diffraction peaks matching the **wurtzite structure.**

The combination of these techniques confirms that Pteris-derived phytochemicals successfully mediate the green synthesis of ZnO NPs, offering an eco-friendly and cost-effective alternative to chemical synthesis routes.(90)

2.12. Mechanisms of Antibacterial Activity of Zinc Oxide Nanoparticles Synthesized Using Pteris: Zinc oxide nanoparticles (ZnO-NPs) created using Pteris plant extracts have shown strong potential as antimicrobial agents. These green-synthesized nanoparticles are effective against a wide range of harmful microbes, including:

- Gram-positive bacteria like ***Staphylococcus aureus*** & ***Bacillus subtilis***
- Gram-negavites bacteria like ***Pseudomonas aeruginosa*** and ***E.coli***
- Fungal pathogens like ***Candida albicans***
- Even strains of bacteria are also become resistant to many types of antibiotics

The powerful antibacterial action of these ZnO-NPs is believed to work through a combination of mechanisms:

a) **Breaking Down Bacterial Membranes:** ZnO-NPs stick to the surface of bacteria, disturbing their protective outer layers. It's the reason of the cell membranes to become leaky and leading to the loss of vital cell contents and finally cells becomes death.
b) **Creating Harmful Reactive Oxygen Species (ROS):** These nanoparticles can trigger the formation of reactive molecules such as **superoxides** and **hydroxyl** radicals, which attack and damage the internal structures of microbes—including fats, proteins, and DNA.
c) **Releasing Zinc Ions (Zn^{2+}):** When ZnO-NPs release zinc ions, these ions can enter microbial cells and interfere with important enzyme activities and **metabolic functions**, further weakening or killing the bacteria.
d) **Disrupting DNA and Protein Synthesis:** By damaging genetic material and essential proteins, ZnO-NPs prevent bacteria from growing and reproducing, effectively halting infections from spreading.

Studies have shown that ZnO-NPs synthesized using **Pteris extracts** may have enhanced antibacterial effects due to the presence of natural bioactive compounds in the plant. These

compounds can either add their own antimicrobial effects or help the nanoparticles bind more effectively to microbes, making the treatment more efficient.

2.13. The Impact of Synthesis Parameters: The efficiency, stability, size, and biological effectiveness of zinc oxide nanoparticles (ZnO-NPs) synthesized using Pteris extracts are highly influenced by various synthesis conditions. These parameters directly affect the nanoparticles physicochemical properties and their subsequent applications. Key factors include:

2.13.1. Zinc Salt Concentration: The amount of zinc precursor (such as **zinc acetate** or **zinc nitrate** or **zinc sulphate**) used during synthesis plays a critical role. Higher concentrations can lead to the formation of larger and more aggregated ZnO particles, which may reduce their surface activity and **antibacterial effectiveness**.

2.13.2. Pteris Plant Extract Volume: The concentration of Pteris extract determines the availability of phytochemicals that act as **reducing** and **stabilizing agents**. An optimal plant-to-metal salt ratio is essential for efficient synthesis, proper particle size control, and improved dispersion stability.

2.13.3. pH of the Reaction Medium: The pH level of the synthesis solution help to increases the nanoformation. **Alkaline conditions** (usually pH 8–12) tend to promote the creation of smaller, well-dispersed ZnO-NPs with enhanced **crystallinity** and better antibacterial performance, while acidic conditions may lead to incomplete reactions or irregular shapes.

2.13.4. Temperature and Reaction Duration: Elevated temperatures enhance the rate of chemical reductions and nucleation, leading to the faster synthesis and more uniform particle formation. Reaction time also plays a crucial role—insufficient time may result in incomplete nanoparticle formation, while excessive time can cause unwanted aggregation. (91)

2.14. Uses and Comparative Efficiency: Comparative analyses indicate that zinc oxide nanoparticles (ZnO-NPs) synthesized using Pteris extracts frequently demonstrate improved **biocompatibility**, **antimicrobial performance**, and environmental friendliness compared to their chemically synthesized counterparts. Their eco-

friendly origin and reduced cytotoxicity make them suitable for a broad range of practical applications, including:

- **Antibacterial coatings** is for the wound dressings and the surgical tools
- **Disinfection agents** in water purification systems
- **Topical antimicrobial** is apply for skin infections and inflammation
- **Slow-release platforms** for drug delivery, especially for antibiotics and anti-inflammatory drugs
- **Biofertilizers** or **nano-pesticides** in sustainable agriculture

Research evidence, including recent in vivo and in vitro studies, suggests that Pteris-based ZnO-NPs can accelerate wound healing and suppress microbial growth more effectively due to the synergistic interaction between ZnO and the plant's bioactive compounds. Their capability to produce **reactive oxygen species (ROS)** and disrupt microbial membranes enhances their effectiveness even against resistant strains.(92)

Chapter: 3

Methods & Materials:

3.1 Objective:

Our main objective of the dissertation work is;

- **Isolate and purify of nanoparticles from the pteris extract**
- **Characterization of nanoparticles by UV-Visible Spectrophotometry, Dynamic Light Scattering analyzer, XRD, and FTIR.**
- **Identify the antimicrobial activity of nanoparticles by Agar Diffusion method and Broth Dilution Assay.**

3.2 Materials:

The chemicals **Zinc sulphate** ($ZnSo_4$), Plant extract, Distilled water, **NaOH**, **HCL**, **Nutrient broth** (NB), **Luria-Bertani broth** (LB**), Iodonitrotetrazolium chloride** (INT dye), DMSO**, KBr.**

3.3 Sample collection:

Fresh plant material was collected from the **South 24 Parganas** district, specifically in and around the area near **Dinabandhu Andrews** College, located in **Garia, Kolkata, West Bengal, India (Latitude: 22.4491° N, Longitude: 88.3915° E)**. The nearby region includes localities such as **Boral, Baishnabghata, Narendrapur, and New Garia**, which are known for their mix of urban and semi-natural green spaces. These areas host diverse plant species and offer suitable conditions for the natural growth of ferns and other flora.

The collection was performed manually during the daytime under suitable weather conditions. Healthy and mature plant specimens were carefully selected to ensure maximum **phytochemical content** and **biological activity**. Immediately after harvesting, the plant material was placed in sterile, labeled polythene bags and transported to the laboratory under ambient conditions to maintain freshness and prevent degradation. All necessary precautions were taken to avoid contamination during the collection and transport processes.

Fig 3.1: Pteris plant

3.4 Sample Identification:

The collected plant specimen was taxonomically identified with the assistance of experienced faculty members from **the Department of Botany at Dinabandhu Andrews College, Garia, Kolkata, West Bengal, India (Pin – 700084)**. The identification process involved careful examination of morphological features and comparison with standard botanical references and herbarium specimens. To ensure authenticity and reproducibility, the identified specimen was assigned a unique voucher number and preserved in **the Herbarium of the Botany Department at Dinabandhu Andrews College**. This voucher specimen will serve as a reference for future studies and verification.

3.5 Solution preparation:

To prepare a **0.1 M** zinc sulphate ($ZnSO_4$) solution, accurately **weigh 28.75 g of zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$, molar mass 287.54 g/mol)** using a digital balance. Dissolve the weighed salt in approximately 800 mL of distilled water in a beaker or volumetric flask, stirring until it is completely dissolved. After the dissolving transfer it into the solution to a 1-liter volumetric flask if needed, and then add distilled water to make sure the final volume will be become exact 1 liter. Mix the solution thoroughly to ensure uniformity.(93)

Fig 3.2: $ZnSo_4$ solution

3.6 Plant Extract Preparation:

Fresh **Pteris** leaves were collected and thoroughly rinsed to eliminate dust and other surface impurities. Initially, the leaves were washed with tap water to remove visible dirt, followed by rinsing with distilled water to eliminate any remaining surface contaminants. After cleaning, approximately **35 grams** of the leaves were weighed using a digital balance. The cleaned leaves were then crushed using a mortar and pestle, with a small amount of distilled water added to facilitate the grinding process. The resulting paste was transferred into a beaker, and the aqueous mixture was gently heated on a magnetic stirrer hot plate at a temperature of approximately **70–80 °C** for 30 minutes. This heating process facilitated the extraction of bioactive compounds from the plant material.

After the heat process, the mixture was then allowed to cool at the room temperature. It was then filtered through Whatman No.1 filter paper to remove any residual solid plant material. The clear filtrate obtained was collected and stored in amber-colored bottles to prevent any type of light-induced degradation of the phytochemicals. The extract was then store at **4** °C until further use.

This prepared Pteris leaf extract served as a natural reducing and stabilizing agent in the green synthesis of zinc oxide (ZnO) nanoparticles, following established procedures (94, 95).

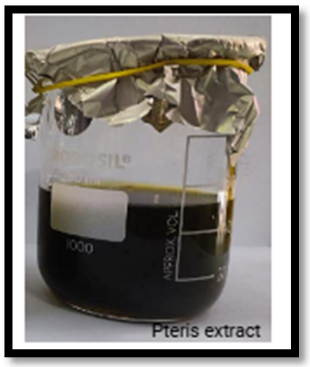

Fig 3.3: pteris extract

3.7.Green Synthesis of Zinc Oxide Nanoparticles (ZnO-NPs) formation method: The zinc oxide nanoparticles were synthesized by using a simple and eco-friendly green synthesis approach.

- At first, a 0.1 M solution of **zinc sulfate ($ZnSO_4 \cdot 7H_2O$)** was freshly prepared (mandetory) by dissolving the required amount of the salt in distilled water.
- To carry out the synthesis, 90 mL of this **zinc sulfate** solution was poured into a clean beaker, and 10 mL of the **Pteris leaf extract** was slowly added while continuously stirring the mixture with a magnetic stirrer. The entire process was conducted at a controlled temperature of around 60–80 °C for 1 to 2 hours. During this time, a gradual change in the color and clarity of the solution was observed, indicating the beginning of nanoparticle formation. (96)
- As the reaction progressed, a white precipitate started forming at the bottom of the beaker, confirming the synthesis of ZnO nanoparticles. Once the reaction was geting finished, the mixture was allowed to cool down to room temperature. The nanoparticles were then separated by centrifugation at **10,000 rpm** for about **15 minutes.** To ensure purity, the collected precipitate was washed several times with distilled water and ethanol, removing any leftover impurities or plant residues.
- Finally, the washed nanoparticles were dried in a hot air oven at 60 °C for 6–8 hours to obtain fine **ZnO nanoparticle powder**. The dried product was carefully stored in airtight containers for further testing and characterization.

This green synthesis technique relies entirely on the natural phytochemicals present in the Pteris extract, which act as both reducing and stabilizing agents. It offers a sustainable and non-toxic alternative to traditional chemical methods and aligns well with environmentally friendly research practices. (97)

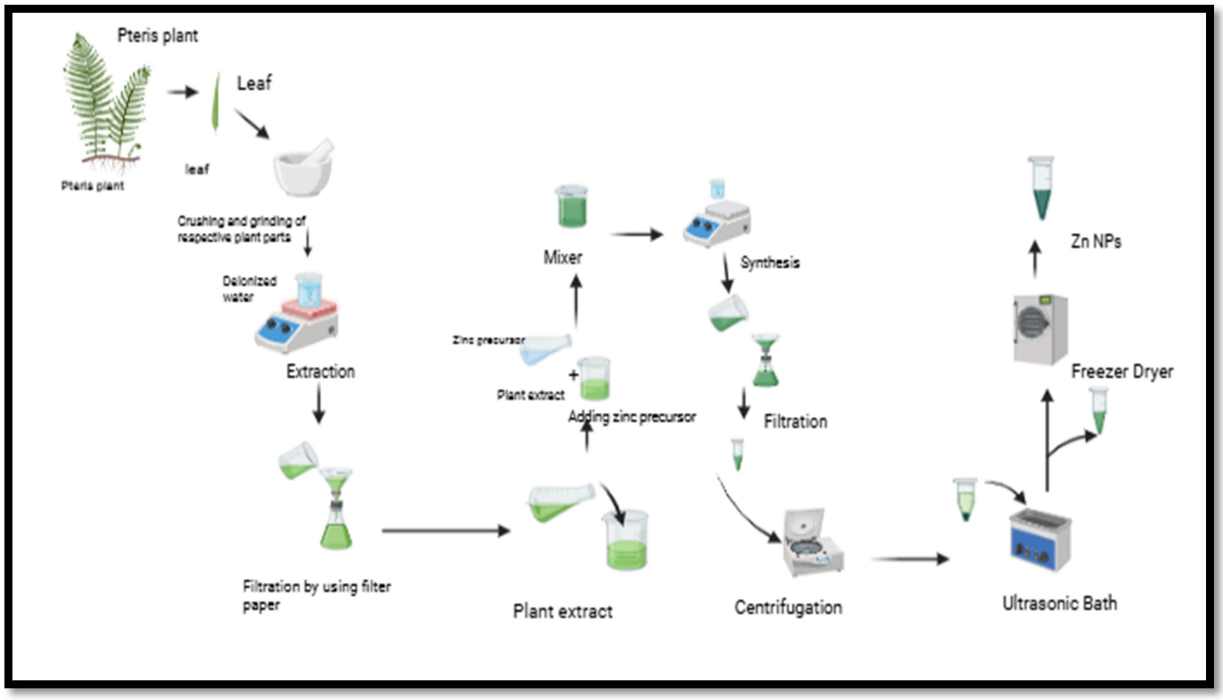

Fig 3.4: Complete process of Zn NPs synthesis

3.8 Characterization:

3.8.1 Characterization of Zinc Oxide Nanoparticles: To better understand the properties of the green-synthesized zinc oxide nanoparticles, a series of standard characterization techniques were carried out. **UV–visible spectroscopy** was first used to confirm the formation of nanoparticles by detecting their typical optical absorption pattern. (98) To identify the plant-based functional groups involved in **reducing** and **stabilizing** the nanoparticles, FTIR analysis was performed. The crystal structure and purity of the **ZnO-NPs** were examined by using **X-ray diffraction method.** Additionally, **dynamic light**

scattering (DLS) was used to determine the average particle size and distribution in solution, offering insight into their stability in a colloidal state. These methods together provided a comprehensive understanding of the synthesized nanoparticles, following approaches similar to those described in earlier studies (99).

3.8.1.1 UV-Visible Spectroscopy of synthesized nanoparticles:

UV–Visible spectroscopy was utilized as a standard analytical technique to confirm the biosynthesis and evaluate the optical properties of zinc oxide nanoparticles (ZnO-NPs) synthesized using Pteris leaf extract. This non-destructive method is widely applied in nanoparticle research due to its effectiveness in monitoring **the electronic transitions** and **surface plasmon resonance (SPR)** behavior characteristic of metal and metal oxide nanoparticles. For spectral analysis, the synthesized ZnO-NPs colloidal solution was subjected to examination without dilution. The measurements were carried out using a **calibrated double-beam UV–Vis spectrophotometer** equipped with quartz cuvettes having a **1 cm path length**. Before sample scanning, baseline correction was performed using distilled water to ensure accuracy.(100) The absorption spectra were recorded across a wavelength range of 200 to 800 nm at ambient room temperature. The scanning range was chosen based on established literature, as it encompasses the region where ZnO nanoparticles typically exhibit a pronounced absorbance peak corresponding to their **band gap energy**. Identification of this peak provides supportive evidence for nanoparticle formation and insights into the size and morphology of the synthesized material. The methodology adheres to widely accepted protocols for green nanoparticle characterization, as reported in previous studies involving plant-mediated ZnO-NPs synthesis . This approach ensures methodological consistency and scientific reliability in characterizing biogenically synthesized nanoparticles. (101)

3.8.1.2 Particle size measurement by dynamic light scattering (DLS) experiment:

Dynamic Light Scattering (DLS) was employed to assess the average hydrodynamic size and particle size distribution of the zinc oxide nanoparticles (ZnO-NPs) synthesized via the green route using **Pteris leaf extract**. This technique analyzes the Brownian motion of suspended nanoparticles and measures fluctuations in the intensity of scattered light to estimate particle size in a colloidal system. (102) The nanoparticle suspension was first filtered using a **0.22 µm membrane filter** to eliminate any dust or large

agglomerates. The filtered sample was then transferred to a clean, dust-free polystyrene cuvette for measurement. The DLS analysis was performed using a particle size analyzer equipped with a **633 nm laser source** at a fixed scattering angle of **90°**, under room temperature conditions. The analysis provided two key parameters: the **Z-average diameter**, which reflects the mean hydrodynamic size of the particles, and the **polydispersity index (PDI),** indicating the distribution uniformity within the nanoparticle suspension. A narrow PDI signifies good colloidal stability and uniformity in particle size, which are crucial for subsequent applications. The **DLS methodology** followed standardized procedures commonly adopted in studies involving the green synthesis and colloidal characterization of metal oxide nanoparticles.(103)

3.8.1.3 **Analyses of the nanoparticles by the help of X-ray diffraction (XRD) methods:**

To investigate the crystalline structure and confirm the phase identity of the zinc oxide nanoparticles (ZnO-NPs) synthesized using Pteris leaf extract, **X-ray Diffraction (XRD)** analysis was performed. XRD is a well-established technique for determining how atoms are arranged within **crystalline materials**, making it particularly useful for analyzing nanoparticles. For the analysis, the dried ZnO nanoparticle powder was gently ground to achieve a uniform consistency and then carefully spread onto a clean XRD sample holder. Ensuring a smooth and even surface helped minimize background noise and improve data clarity. The sample was scanned using an X-ray **diffractometer with copper K-alpha (Cu-Kα) radiation**, operating at a wavelength of **1.5406 Å**. The machine was set to **40 kV** and **30 mA**, with the scan conducted over a **2θ range of 20° to 80°, using a step size of 0.02°**. These settings allowed for accurate detection of diffraction peaks. The observed peaks were then compared to the standard reference data by the Joint Committee on Powder Diffraction Standards (JCPDS) to identify the crystal structure of the all measured nanoparticles. The presence of well-defined peaks corresponding to the **hexagonal wurtzite structure** of ZnO confirmed successful synthesis and crystallinity of the product. (104)

3.8.1.4 **Fourier transform infrared spectroscopic (FTIR) analysis:**

FTIR spectroscopy was used to identify which functional groups were present in the

Pteris leaf extract and to understand their role in the reduction and stabilization of zinc oxide nanoparticles (ZnO-NPs). This technique is especially useful for detecting specific **chemical bonds** and **functional groups** based on their characteristic absorption of infrared light. To begin the analysis, a small quantity of the synthesized and dried ZnO nanoparticle powder was mixed with spectroscopic-grade **potassium bromide (KBr)** in a **1:100 ratio** by weight. This mixture was then finely ground using a clean mortar and pestle to ensure even blending. The resulting powder was then compressed into a transparent pellet by using a hydraulic press under high pressure. The FTIR spectra were recorded using an FTIR spectrometer in the range of **4000 to 400 cm^{-1}** at room temperature. The background spectrum was measured using a pure KBr pellet, which was automatically subtracted from the sample spectra to eliminate interference. The main absorption peaks were carefully analyzed to determine the functional groups involved, particularly those from the plant extract that may have acted as capping and stabilizing agents during nanoparticle synthesis.(105)

Sl. No.	FTIR spectral region (cm^{-1})	Assigned functional groups
1	749-500	R-C-H (106)
2	941-803	C–H in plane deformation with aromatic- ring stretching (107)
3	1090-1049	-C-O-C- (108)
4	1260-1100	Amide III/ N-H bending and the (C-N) stretching vibration (109)
5	1380-1330	CH_3 deformation (110)
6	1490-1401	Symmetrical stretching vibrations of –COO (111)
7	1580-1510	Amide II/ N-H bending vibration (112)
8	1700-1600	Amide I/ C=O stretching vibration (113)
9	2973-2541	CH_3/ C-H stretching vibration (114)
10	3450-3250	N-H stretching (115)
11	3600-3200	O-H stretching of alcohols or phenols (116)

Table 3.1:**List of different FTIR spectral regions with their assigned functional groups.**

3.8.2 Antimicrobial Activity of Zinc nanoparticle: To assess the antibacterial potential of the biosynthesized ZnO nanoparticles, two widely accepted microbiological assays were conducted: **the agar well diffusion methods** and **the broth dilution methods**. These tests helped determine how effectively the nanoparticles inhibited bacterial growth. In the agar dilution method, different concentrations of ZnO nanoparticles were mixed into LB agar media, and bacterial cultures were spread on the surface. After incubation, any visible inhibition of bacterial growth was noted as a sign of antimicrobial activity. For a more quantitative analysis, the broth dilution method was employed using LB broth supplemented with p-iodonitrotetrazolium violet (INT) dye, which changes color in the presence of metabolically active bacteria. By visualized the dye's colour shift, the minimum inhibitory concentration (MIC) of the ZnO-NPs was determined—this being the lowest concentration capable of preventing visible bacterial population. These procedures followed standard protocols as described in earlier research on nanoparticle antimicrobial testing (117), and they provided a reliable measure of the synthesized ZnO-NPs' antibacterial effectiveness.

3.8.2.1 Agar well diffusion methods analysis:

To assess the **antibacterial potential** of the biosynthesized zinc oxide nanoparticles (ZnO-NPs), an agar well diffusion method was carried out using two model bacterial strains: ***Bacillus subtilis*** **(Gram-positive)** and ***Escherichia coli*** **(Gram-negative).** Nutrient agar served as the culture medium and was prepared according to the standard manufacturer's instructions. Once the medium was sterilized by autoclaving at 121°C for 15 minutes, it was poured into sterile Petri dishes and allowed to solidify in a clean environment. Fresh overnight bacterial cultures were grown in nutrient broth and adjusted to a turbidity equivalent to the **0.5 McFarland standard**. By the help of sterile cotton swabs all cultures were then evenly spread into the surface of the solidified agar to make clear a consistent microbial lawn. (118) After inoculation, four wells (**each 10 mm in diameter**) were carefully made in the agar using a sterile cork borer. Three of the wells were filled with 50 µL of ZnO-NP solutions at concentrations of 25, 50, and 100 µg/mL, respectively. The remaining well were acted as a negative control and was filled with 50 µL of non-ionized distilled water to verify that any antibacterial activity observed was

due to the nanoparticles alone. The plates were then incubated in an inverted position at **37 ± 1°C for 24 hours**. After incubation, the plates were examined for clear zones around the wells, which indicate bacterial growth inhibition. (119). To make sure the perfect result, each test was conducted in triplicate.

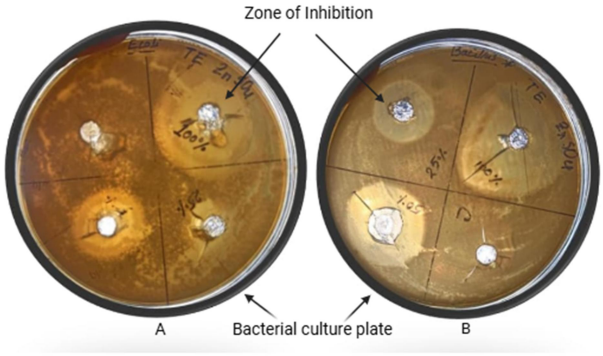

Fig 3.5: Bacterial culture plates treated by ZnNPs solution; A) *Escherichia coli* and B) *Bacillus subtilis*

3.8.2.2.Broth micro dilution assay:

The antibacterial efficacy of the green-synthesized zinc oxide nanoparticles (ZnO-NPs) was further evaluated using a broth micro dilution method against ***Staphylococcus aureus*** (Gram-positive) and ***Escherichia coli*** (Gram-negative). **Luria-Bertani** (LB) broth was applied as the culture medium for this assay. (120) Each well of a sterile 96-well microtiter plate was set up to contain a total volume of 200 µL. For all treatments, 20 µL of fresh bacterial inoculum—standardized to match **0.5 McFarland turbidity**—was used. The remaining 180 µL consisted of varying proportions of LB broth and ZnO-NPs solution, depending on the nanoparticle concentration being tested. The nanodose concentrations used were **0.25, 0.5, 0.75, 1.0, and 1.25 mg/mL** . For the control sets, **180 µL** of LB broth was mixed with the 20 µL of bacterial inoculum, with no nanoparticles

added. For treated wells, the nanoparticle solution was adjusted to the desired concentration, and the volume of LB broth was reduced accordingly to maintain a constant total volume of 200 µL. The plates were incubated overnight at 37 ± 1°C. Following incubation, 20 µL of an **INT (iodonitrotetrazolium)** dye solution was added to each well as a colorimetric indicator of bacterial viability. The plates were then kept in the dark for an additional 30 minutes to allow proper dye reaction. Any color change was observed visually to assess microbial growth inhibition. All treatments were performed in triplicate to ensure consistency and reproducibility. (121).

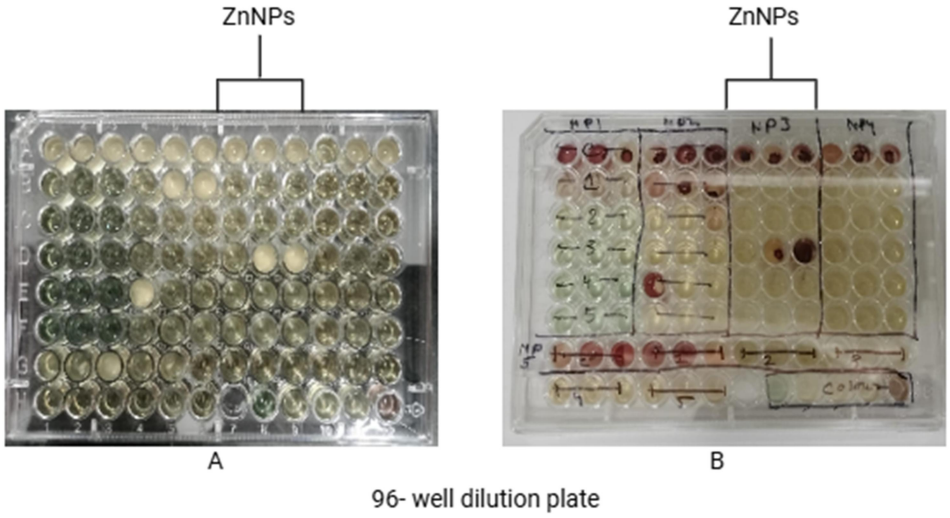

Fig 3.6: Bacterial culture treatment under 96- well dilution plate ; A) Overnight incubated plate, B) 30 mins dark incubated plate after apply INT dye.

Chapter: 4

Results and discussions

4.1.In vitro Green Synthesis of Zinc Nanoparticles:

Zinc nanoparticles were synthesized in vitro using an eco-friendly approach by exposing Pteris extract to a 0.1 M aqueous solution of zinc sulphate ($ZnSO_4$). Upon the addition of the plant extract to the metal salt solution, a time-dependent color change was observed from dark green to a light yellowish-green hue. This color shift indicated the successful biosynthesis of zinc nanoparticles (ZnO-NPs), suggesting the **reduction of Zn^{2+}** ions and the formation of **stable nanoparticles**. The visual change in the reaction mixture is attributed to the excitation of **surface plasmon resonance (SPR)**, which typically accompanies the formation of metal nanoparticles.

No color change was observed in either the zinc sulfate solution (negative control) or the **Pteris extract alone (positive control)**, confirming that the color shift was due to nanoparticle synthesis and not due to any inherent property of the individual components.

Similar biosynthetic approaches have been reported earlier using different biological sources. For instance, Ramesh et al. (2014)(122) reported a comparable green synthesis of ZnO-NPs utilizing ***Calotropis gigantea*** leaf extract, where a noticeable color change indicated the formation of nanoparticles. Likewise, Vijayaraghavan et al. (2012)(123) successfully employed ***Aspergillus flavus*** for the biosynthesis of zinc oxide nanoparticles, observing similar visual cues during nanoparticle formation. In another study, Bhuyan et al. (2015)(124) demonstrated the synthesis of ZnO-NPs using ***Azadirachta indica*** leaf extract, reinforcing the efficacy of plant-based systems for nanoparticle synthesis.

These findings align with our current observation that plant extracts, due to their rich phytochemical composition (including flavonoids, terpenoids, and polyphenols), act as both reducing and stabilizing agents in nanoparticle synthesis. The simplicity, cost-effectiveness, and environmental compatibility of this method position it as a promising alternative to traditional chemical synthesis methods.

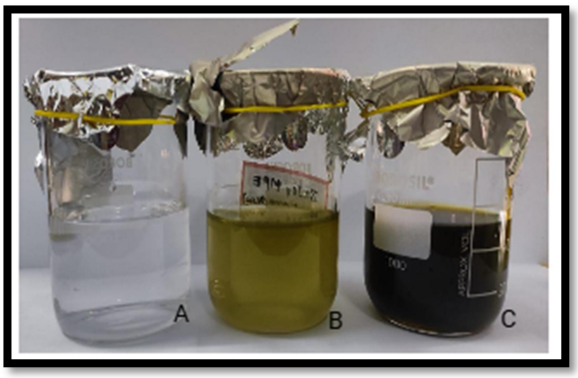

Fig: 4.1 Visual change in colour of the reaction mixture compared with the control set; A) Zinc sulfate solution; B) reaction mixture; C) Pteris extract.

4.2. Characterization of the Zing nano particles:

4.2.1. UV-Visible Spectrophotometric Analysis of Zinc Nanoparticles Synthesized Using Pteris Extract:

To confirm the formation of zinc nanoparticles, the reaction mixture—where Pteris plant extract was exposed **to 0.1 M zinc sulphate solution**—was analyzed using UV-Visible spectrophotometry. A noticeable color change from dark green to a light yellowish-green was observed, which visually indicated that zinc nanoparticles had likely formed. The UV-Vis spectrum of the samples was recorded between 300 to 800 nm using a **GENESYS 50 spectrophotometer**. All three samples- OD culture 1, OD culture 2, and OD culture 3—showed absorption peaks at around **425 nm** with absorbance values of 0.367, 0.366, and 0.366, respectively. Another minor peak was observed around 675 nm, with absorbance values ranging from 0.217 to 0.221 [Fig: 4.2]. Although zinc nanoparticles don't usually produce a very **sharp surface plasmon resonance (SPR)** peak like silver or gold, the broad absorption at 425 nm suggests that nanoparticles were successfully synthesized. This is likely due to the movement of electrons on the surface of the zinc particles, which is influenced by their size, shape, and the presence of bioactive compounds from the Pteris extract acting as stabilizers.

Sample	OD culture 1	OD culture 2	OD culture 3
Absorbance (425)	**0.367**	**0.366**	**0.366**
Absorbance (675)	**0.217**	**0.219**	**0.221**

Table 4.1 : UV- visible spectra observed data.

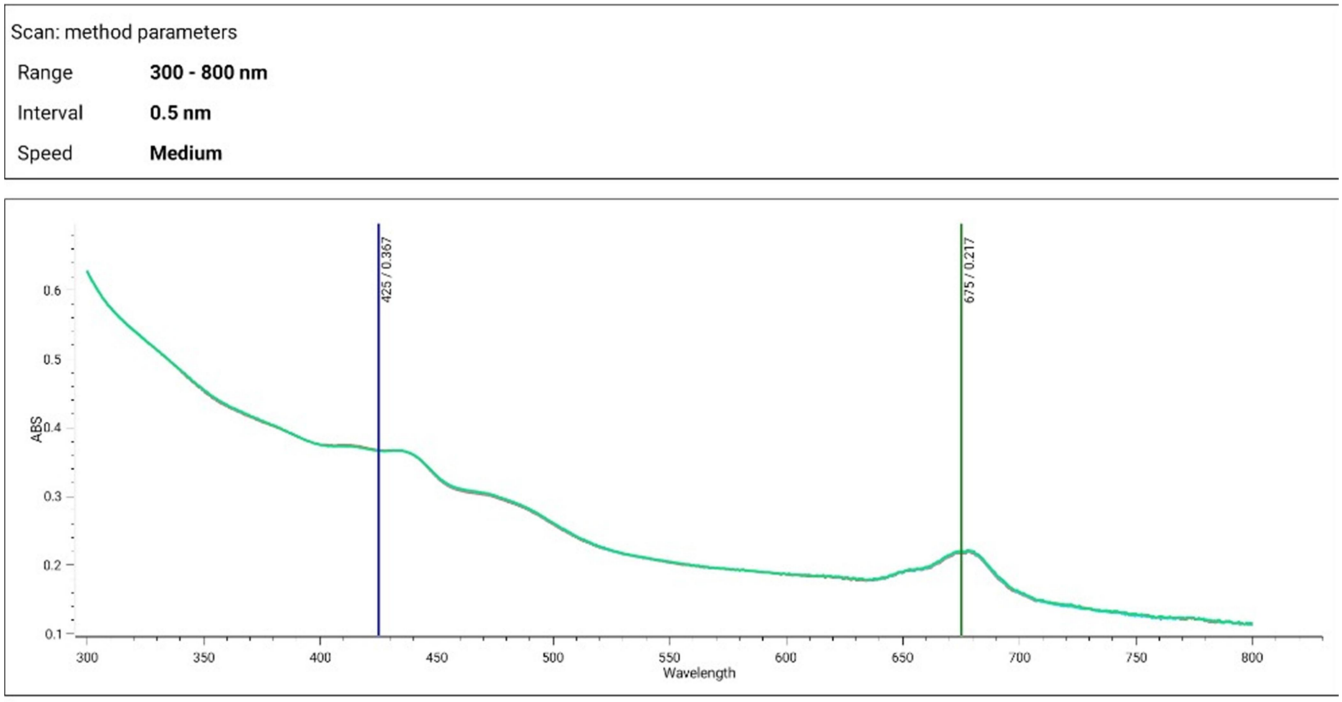

Fig. 4.2 UV-Vis spectra showing SPR peak at 425 nm for all samples, confirming ZnO-NPs formation.

Our findings are in line with previous studies. For instance, researchers like Ramesh et al. (2014)(125) and Suresh et al. (2015)(126) also reported similar absorbance ranges when using plant and fungal sources for the green synthesis of zinc nanoparticles. Overall, the UV-Vis results confirm that Pteris extract effectively aided in the green synthesis of zinc nanoparticles. The consistent absorption values across samples also suggest that the nanoparticles were stable and well-dispersed, likely due to natural capping agents in the extract.

4.2.2. DLS Analysis:

The DLS (Dynamic Light Scattering) measurement was performed to determine the particle size distribution of the green-synthesized zinc nanoparticles . The analysis revealed that the nanoparticles were fairly mono dispersed in nature, with a size distribution ranging between **20–150 nm**. That's indicating effective capping and stabilization by the phytochemicals present in the ***Pteris* extract**.

These findings are consistent with earlier reports on biogenic nanoparticle synthesis. For instance, Saha et al. (2010)(127) synthesized silver nanoparticles using the fungus *Bipolaris nodulosa*, with sizes ranging from 10–60 nm. Similarly, Jyoti et al. (2016) (128)reported the formation of silver nanoparticles in the range of 19–53 nm, while Husseiny et al. (2015)(129) synthesized silver nanoparticles using *Fusarium oxysporum* within a similar size distribution. The slightly higher particle size observed in the current study may be due to the intrinsic properties of zinc and the natural biomolecules from the *Pteris* extract acting as reducing and stabilizing agents.

4.2.3. XRD analyses of Zinc oxide Nanoparticles:

The XRD pattern of the green-synthesized ZnO nanoparticles by using Pteris extract is shown in Fig [4.3]. The diffraction data was taken in the 2θ range of 10° to 70°.

The pattern exhibits the following key features:

- A broad hump is observed between 20° and 35° (2θ), with no sharp or well-defined diffraction peaks.
- The overall intensity was get decreases by gradually with increasing 2θ.
- The absence of distinct crystalline peaks indicates that the sample lacks long-range crystallinity.

These observations suggest that the synthesized ZnO nanoparticles are predominantly amorphous or poorly crystalline in nature. The broad hump in the XRD pattern is characteristic of materials with short-range order, typical of amorphous structures or ultra-small nanocrystalline domains. (130)

Such structural features are often seen in green-synthesized nanoparticles, where the use of plant extracts (in this case, Pteris extract) provides a biological reduction and stabilization environment that may hinder full crystallization. The presence of organic

molecules such as flavonoids, polyphenols, and other phytochemicals in the extract can act as capping agents, leading to restricted crystal growth and resulting in either an amorphous matrix or extremely fine nanocrystals.

Moreover, the lack of a thermal post-treatment step (such as calcination or annealing) likely contributes to the preservation of the amorphous nature of the sample. If a more crystalline structure is desired, annealing at elevated temperatures (e.g., 400–600°C) could be employed to enhance crystal growth and improve phase purity.(131)

Overall, the XRD results confirm the formation of amorphous or nanocrystalline ZnO nanoparticles, stabilized by Pteris extract, which is consistent with typical outcomes of green synthesis methods.

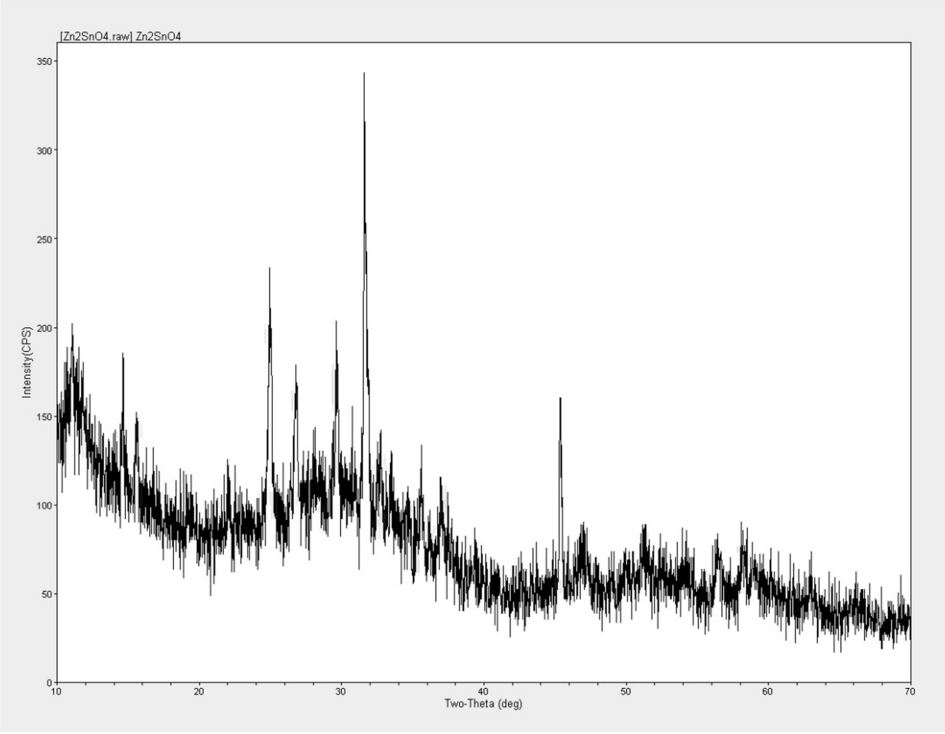

Fig 4.3: Representative XRD pattern of Zinc nanoparticles.

4.2.4. FTIR Analyses of Zinc Nanoparticles:

FTIR measurements were conducted to identify the possible interactions between zinc ions and Pteris extract during the green synthesis of zinc nanoparticles. Figure (4.4) displays the FTIR absorption spectra of the Pteris extract (Spectrum B) and the synthesized zinc nanoparticles (Spectrum A). The comparison of these spectra reveals several characteristic absorption bands which suggest the involvement of functional groups from the Pteris extract in the reduction and stabilization of zinc nanoparticles.

Both spectra exhibited a broad band around **3,420–3,440 cm^{-1},** attributed to O–H stretching vibrations of hydroxyl groups, indicating the presence of phenolic compounds and flavonoids—common secondary metabolites in Pteris species. These compounds are known to act as reducing agents in the biosynthesis of metal nanoparticles (Jin and Bai, 2002; Socrates, 2004).(132;133)

The peaks near **2,920 cm^{-1}** correspond to **C–H stretching vibrations**, which are typically associated with aliphatic hydrocarbon chains. This suggests the presence of long-chain organic molecules such as terpenoids or fatty acids.

A significant band appeared around **1,630–1,650 cm^{-1},** representing amide I **(C=O stretching)** from proteins or peptides. This indicates that proteinaceous compounds in the extract may have bound to the nanoparticle surface, serving as capping agents (Barth, 2007). In addition, a band around 1,380–1,450 cm^{-1} is associated with symmetric **$–COO^-$** stretching, also found in amino acids and organic acids.

Another noticeable band at approximately **1,070 cm^{-1}** corresponds to **C–O–C or C–O stretching**, which is characteristic of alcohols, esters, and polysaccharides. These components are also abundant in plant extracts and may contribute to both reduction and stabilization processes.

Importantly, the fingerprint region between **500 and 750 cm^{-1}** exhibited subtle peaks indicating bending vibrations associated with R–CH groups, possibly from poly-phenolic compounds, which are widely reported in ***Pteris vittata*** and related species (Singh et al., 2010).(134)

According to Lin et al. (2005) (135), carbonyl groups from amino acids and peptides show a high affinity for metal ions and likely played a crucial role in coordinating and stabilizing the zinc nanoparticles. Proteins and other macromolecules could form a bio-

layer on the nanoparticle surface through interactions involving free amine or carboxyl groups (Ninganagouda et al., 2014; Singh et al., 2013) (136). Similar observations were also stydy by Banu et al. (2011),(137) reinforcing the role of bioorganic molecules in nanoparticle stabilization.

Thus, FTIR analysis clearly suggests that various bioactive compounds in Pteris extract—such as flavonoids, phenols, proteins, and organic acids—not only contributed to the reduction of Zn^{2+} to Zn nanoparticles, but also formed a protective capping layer. This biocapping effect is vital for the long-term stability and dispersity of the synthesized zinc nanoparticles.

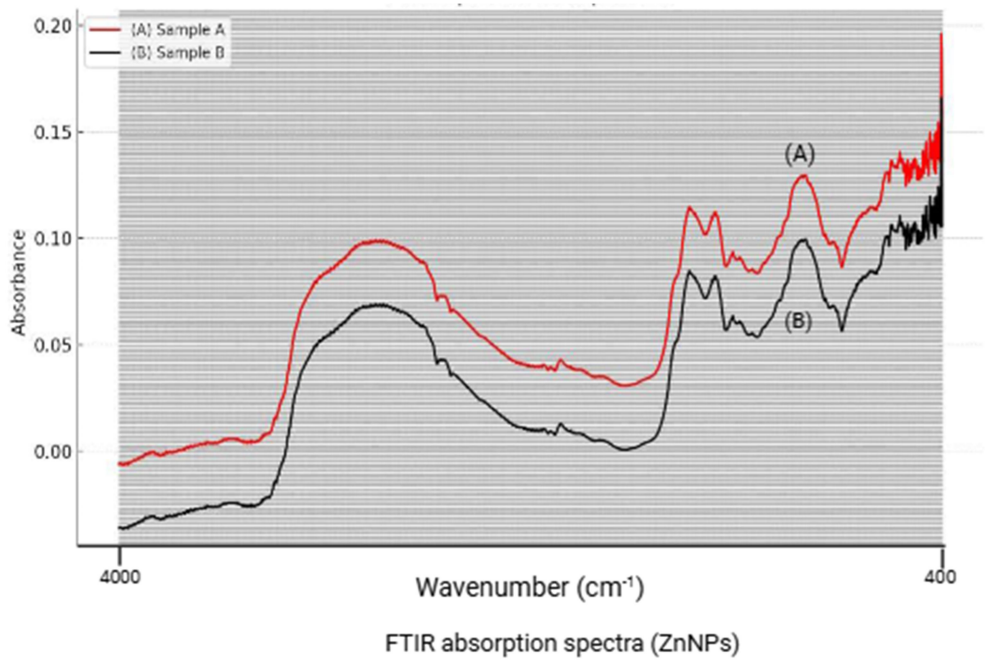

Fig 4.4: FTIR absorption spectra of (A) Zinc oxide nanoparticles and (B) Pteris extract.

4.3. Antimicrobial activity analysis of ZnO-NPs :

4.3.1. Agar diffusion assay:

In this experiment, zinc sulphate ($ZnSO_4$) at a concentration of **0.1 M** was used to synthesize zinc oxide (ZnO) nanoparticles through a green synthesis approach by adding Pteris plant extract. After the nanoparticles formed, the solution was used as-is **(100%)** and also diluted to **50% and 25% concentrations**. These three samples were tested for their antimicrobial activity using the agar well diffusion method, with two nutrient agar plates – one containing ***E. coli*** and the other ***Bacillus subtilis***. A control well (with no nanoparticles) was also included on both plates for comparison.(138)

After overnight incubation, clear zones around the wells showed where bacteria were unable to grow, indicating the effectiveness of the ZnO nanoparticles.(Fig:4.5) The 100% concentration showed the largest zones of inhibition, with both ***E. coli*** and ***Bacillus subtilis*** having a zone of **4.0 cm**. As the concentration was lowered to 50%, the inhibition zones decreased to **2.9 cm** for ***E. coli*** and **3.0 cm** for ***Bacillus***. At 25% concentration, the zones were the smallest — **1.2 cm** for ***E. coli*** and **2.2 cm** for ***Bacillus***. The control wells, as expected, showed no inhibition, confirming that the antibacterial activity was due to the ZnO nanoparticles.

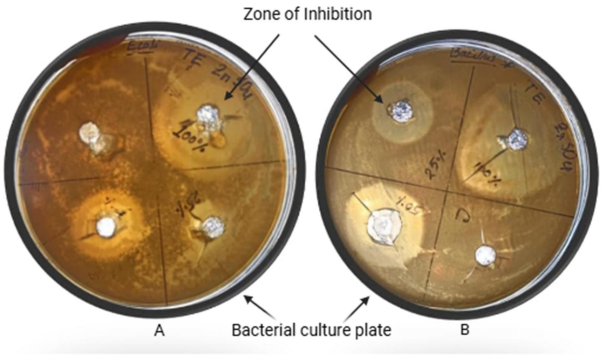

Fig 4.5: Visualizing the zone of inhibition by two microbes; A) ***Escherichia coli*** **and B)** ***Bacillus subtilis***

Overall, the results show a clear dose-dependent antibacterial effect — higher concentrations of ZnO nanoparticles were more effective in stopping bacterial growth. By a graphical analysis Fig: (4.6), ***Bacillus subtilis***, interestingly, appeared to be slightly more sensitive than ***E. coli***, especially at the lower concentrations. (139)

Table 4.2: Zone of Inhibition Data

SL. No.	Name of Nanoparticle	E. coli (cm)	Bacillus (cm)	Zone of Inhibition (cm)	Status (S-R)
1.	$ZnSo_4$+ Pteris Extract.(control)	0	0	E. coli: 0 Bacillus: 0	R
2.	100%	4	4	E. coli: 3.5 Bacillus: 3.5	S
3.	50%	2.9	3	E. coli: 2.4 Bacillus: 2.5	S
4.	25%	1.2	2.2	E. coli: 0.7 Bacillus: 1.7	R

Note:

- S: Sensitive
- R: Resistance
- As zinc nanoparticles concentration increases, the zone of inhibition increases, indicating greater antibacterial effectiveness.

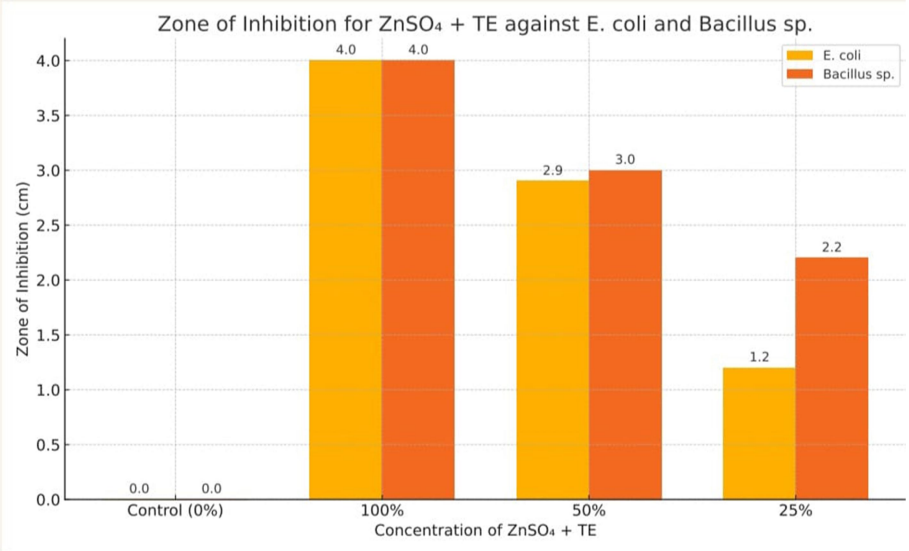

Fig 4.6: Graphical analysis of zone of inhibition by *; E. coli* and *Bacillus sp.*

These findings are in line with other studies that also explored the green synthesis of ZnO nanoparticles. For example, a analysis by Ramesh et al. (2014) used; Aloe vera extract for ZnO nanoparticle synthesis and reported similar antimicrobial effects against both Gram-positive and Gram-negative bacteria, supporting the effectiveness of plant-mediated ZnO NPs. This simple yet eco-friendly method using Pteris extract could be a step forward in developing natural antimicrobial alternatives, especially important at a time when antibiotic resistance is a growing concern.

4.3.2. Broth micro dilution Assay Analysis of ZnO Nanoparticles Synthesized from Pteris Plant:

To determine the antimicrobial efficacy of green-synthesized zinc oxide nanoparticles (ZnO NPs) from Pteris plant extract, a broth micro dilution assay was conducted against two bacterial strains: ***Escherichia coli*** **(Gram-negative)** and ***Staphylococcus aureus*** (**Gram-positive**). The assay tested varying concentrations of ZnO-NPs (0.25–1.25 mM), and the percentage of bacterial growth inhibition was calculated. The results, depicted in Fig: (4.7), revealed a clear dose-dependent reduction in bacterial growth in both strains.

Among the two, ***S. aureus*** demonstrated greater sensitivity, exhibiting up to **83.2%** inhibition at **1.25 mM**, whereas ***E. coli*** showed a lower inhibition of 72.1% at the same concentration. These

findings align with prior studies suggesting that Gram-positive bacteria, with their thick peptidoglycan wall, may have greater surface interaction with metallic nanoparticles, while Gram-negative bacteria, with an additional lipopolysaccharide-rich outer membrane, exhibit comparatively more resistance to nanoparticle penetration and action.

To further investigate the antibacterial mechanism of ZnO- NPs, membrane integrity assays were performed. These included quantification of DNA and protein leakage, which serves as a marker for membrane disruption and cell lysis.

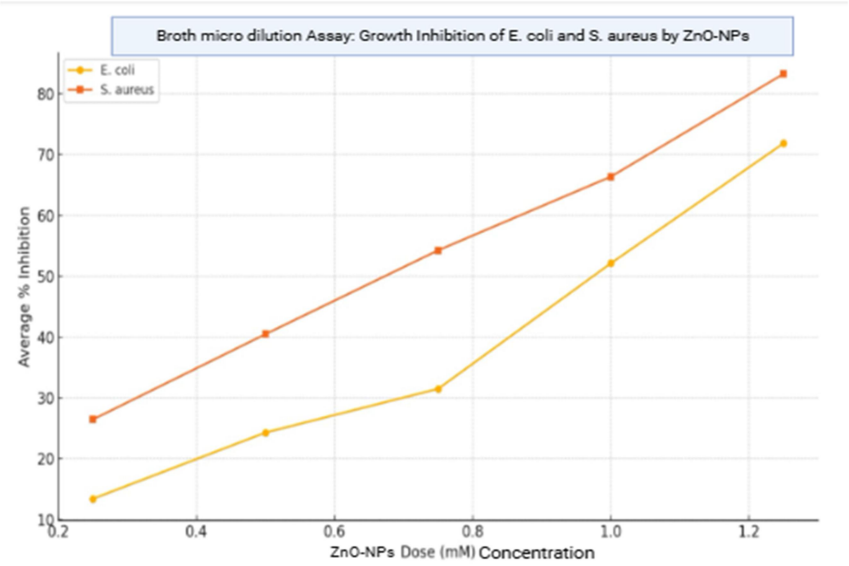

Fig 4.7: Broth micro dilution assay; showing it's growth inhibition percentages of *E. coli* and *S. aureus* treated with ZnO-NPs.

To further investigate the antibacterial mechanism of ZnO- NPs, membrane integrity assays were performed. These included quantification of DNA and protein leakage, which serves as a marker for membrane disruption and cell lysis.

In E. coli, treatment with ZnO NPs synthesized using thermal extract (Zn-TE) resulted in a significant increase in absorbance at **260 nm** (DNA) and 280 nm (protein), indicating leakage of nucleic acids and proteins into the extracellular medium. Specifically, DNA leakage increased from **0.33 (**control) to **0.64**, while protein leakage rose from **0.27 to 0.46**. These results are presented in Fig (4.8).

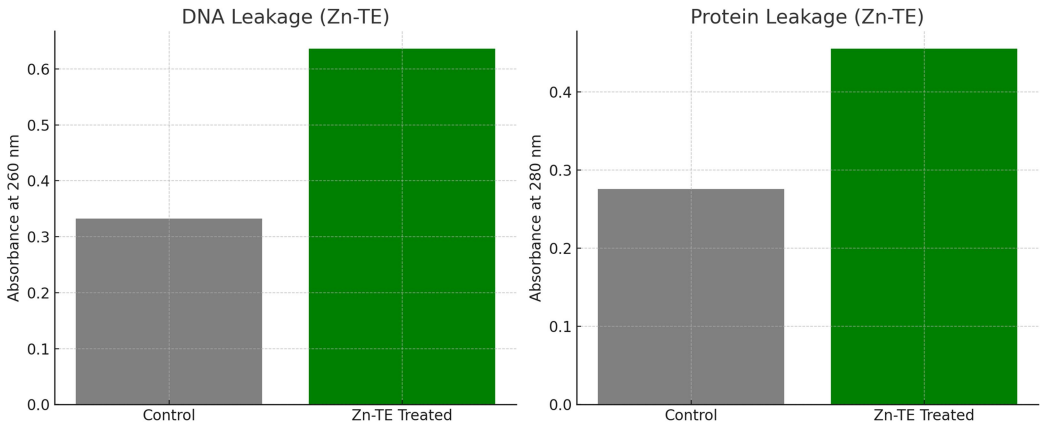

Fig 4.8: DNA **(260 nm)** and protein **(280 nm)** leakage in *E. coli* cells treated with ZnO-NPs

Similarly, in *S. aureus*, ZnO-NPs treatment caused a rise in DNA leakage from 0.28 to 0.58, and protein leakage from 0.22 to 0.42, shown in Fig (4.9). This confirms that ZnO -NPs compromise the bacterial cell membrane, ultimately leading to cell death.

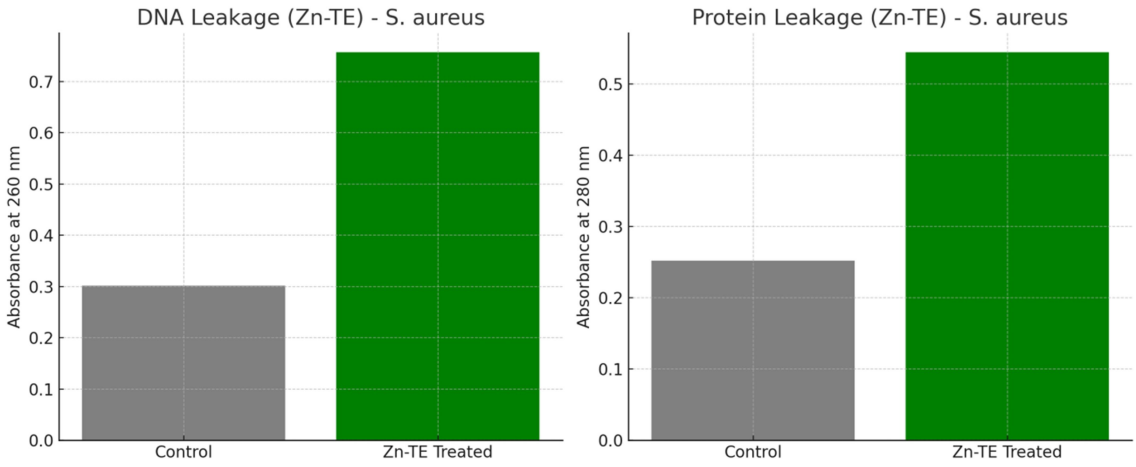

Fig 4.9: DNA **(260 nm)** and the protein leakage **(280 nm)** in *S. aureus* cells treated by ZnO-NPs.

These outcomes are consistent with earlier reports, such as Singh et al. (2021) (140), who observed similar membrane leakage patterns in bacteria treated with neem-synthesized ZnO-NPs, and Chatterjee et al. (2019) (141), who demonstrated nanoparticle-induced leakage in clinical pathogens.

Chapter: 5

Conclusion:

This study successfully explored a green and eco-friendly method to create zinc oxide nanoparticles (ZnO-NPs) using "***Pteris vittata***" plant extract. The natural compounds in the plant acted as both reducing and stabilizing agents, allowing the formation of nanoparticles without the use of harmful chemicals. These all ZnO-NPs were showed a strong antibacterial activity against both **Gram-positive** and **Gram-negative** bacteria. The results from various tests like **UV-Vis spectroscopy, FTIR, XRD,** and **DLS** confirmed the successful synthesis and stability of the particles, reinforcing the idea that **Pteris** can be a powerful and sustainable source for nanoparticle production with potential in medical and environmental fields.

Looking forward, there's a lot of scope to build on this work. Future studies can dive deeper into understanding how these nanoparticles interact with living cells and ensure they are safe for broader use. There's also potential to fine-tune the synthesis process for better results, create practical applications like wound healing gels or antibacterial coatings, and even scale up production for industrial use. Exploring other plants or similar species might also uncover even more effective options for green nanoparticle synthesis.

Chapter: 6

Appendix

Sl. No.	Material/Equipment Used	Specification/Details
1.	Pteris leaves	Fresh, botanically verified
2.	Zinc sulphate ($ZnSo_4$)	0.1 M aqueous solution
3.	Distilled water	Double-distilled
4.	Mortar and pestle	For mechanical extraction.
5.	Whatman No. 1 filter paper	For extract filtration
6.	Magnetic stirrer	For uniform mixing
7.	Conical flasks	For synthesis and incubation
8.	UV-Vis Spectrophotometer	For surface plasmon resonance analysis
9.	FTIR Spectrometer	For functional group analysis
10.	X-ray Diffraction (XRD) Machine	For crystalline structure identification
11.	Dynamic Light Scattering (DLS) Analyzer.	For size distribution analysis
12.	LB Broth/Nutrient Broth	For culturing bacteria
13.	Agar media and Petri dishes	For agar well diffusion tests.
14.	Sterile cork borer	To create wells in agar plates
15.	Incubator	37°C, for bacterial incubation
16.	96-well microtiter plates	For broth dilution assay

List of Bacterial Strains-

Sl. No.	Organism	Type	Gram Staining
1	***Escherichia coli***	**Test organism**	**Gram- negative**
2	***Bacillus subtilis***	**Test organism**	**Gram- positive**
3	***Staphylococcus aureus***	**Test organism**	**Gram- positive**

Spectral peak-

Technique	Observed peaks	Interpretation
UV-Vis	**~425 nm**	**SPR- like peak indicative of ZnO-NP formation**
FTIR	**3600-3200, 1700-1600 nm, 1401-1490, 1100-1260 cm^{-1}**	**-OH, -C=O, -COO^-, -C-N, -NH_2 groups (from pteris)**
XRD	**Broad peak (20-35° 2Θ)**	**Amorphous or nanocrystalline ZnO structures**
DLS	**~20-150 nm size distribution**	**Hydrodynamic diameter of $ZnSo_4$**

Visual observation

- **Color change:** Dark green to yellowish-green- indicates successful ZnO-NPs formation.
- **Plate Images**: Clear zones around wells against ***E.Coli***, ***Bacillus subtilis***, and ***Staphylococcus aureus*** indicate antimicrobial activity.
- **UV-Vis Spectrum:** Peak at **425 nm** confirms nanoparticle synthesis.
- **FTIR Shifts:** Indicate bonding of phytochemicals (e.g., flavonoids, phenolics, saponins) with ZnO-NP surface.

Chapter: 7

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Introduction:
Nanotechnology, or the manipulation of matter at the atomic and molecular level, has revolutionized several scientific fields of study (1), most notably environmental studies, materials engineering, and medicine (2). Among the various nanomaterials being studied, zinc oxide nanoparticles (ZnO-NPs) have attracted significant attention due to their unique physicochemical properties (3). The most notable among these are their strong antimicrobial activity against a wide-ranging of microbes, high surface area-to-volume ratio, excellent photocatalytic performance, UV-blocking ability (4), and robust chemical stability. ZnO-NPs are currently used in a wide range of applications, including sunscreens, cosmetics, antibacterial coatings, food -packaging, water waste treatment (5), and as additives in paints and rubber.(6)
Although zinc-based materials have been used for centuries, the systematic production and documentation of zinc oxide nanoparticles (ZnO- NPs) began much later (7), primarily emerging with the advancement of nanotechnology in the late 20th and early 21st centuries.(8) While ancient societies were not explicitly aware of nanoparticles, they did utilize zinc compounds for various purposes, such as in ointments and medicinal preparations (9). The nanoscale properties of zinc oxide, however, were not fully understood or intentionally applied in historical artifacts like silver or gold nanoparticles (10). The purposeful synthesis and use of ZnO nanoparticles for their unique optical, antibacterial, and catalytic properties is a relatively modern development, driven by advancements in materials science and nanotechnology.
Milestone Contributor Year Significance
Colloidal metal -nanoparticles (Ag) Michael Faraday 1857 Initiated the process for nanoparticle research
Chemical synthesis of silver nanoparticles Creighton et al. 1979 Employing reducing agents, the first modern-day synthesis
Characterization and synthesis of ZnO-NPs Wang et al. (commonly cited) 2004 Demonstrated controlled synthesis and size-dependent properties of ZnO nanoparticles
Table 1.1: Key Milestones in the Development of Nanoparticle Synthesis and Characterization.

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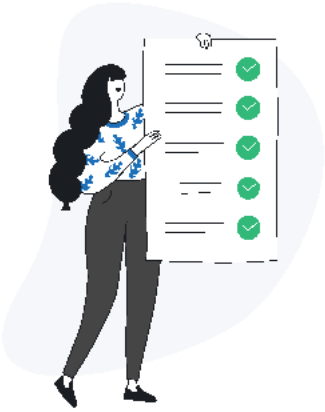
Nanoparticles (NPs) are very small materials with at least one-dimension in the nanometer range, typically between 1 and 100 nanometers (nm). (11) At this scale range, materials exhibit their unique physicochemical abilities due to their high surface area-to-volume ratio, increased surface energy, and quantum confinement effects.(12) These characteristics result in distinct optical, electrical, magnetic, catalytic, and biological properties compared to their bulk counterparts. Nanoparticles can be composed of various materials, including metals (like silver, gold, and zinc), metal oxides (such as TiO_2 and ZnO),(13) polymers, lipids, and ceramics. Among these, zinc oxide nanoparticles also (ZnO- NPs) have gained their significant attention due to their remarkable photocatalytic activity, UV-blocking capability, and strong antimicrobial and anti-inflammatory properties.(14) These features make ZnO-NPs highly valuable in diverse fields such as environmental remediation, agriculture, food preservation, cosmetics, sunscreen formulations, and biomedical applications.(15)

Fig: 1.1 classes of different nano particles.



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Nanoparticles organized them into different categories:
Type of Nanoparticle Composition Essential Characteristics Applications Nanotechnology Relevance
Metal Nanoparticles Silver (Ag), Gold (Au), Copper (Cu) Antimicrobial, Optical (SPR), Conductive Antibacterial agents. Imaging, Sensors Nano-optics, Biomedical nano- sensors
Metal Oxide Nanoparticles ZnO, TiO_2, Fe_3O_4 Photocatalytic, Magnetic, UV- absorbing Sunscreens, MRI contrast, Wastewater treatment Environmental nanotech, Magnetic nanotech
Carbon-Based Nanoparticles CNTs,
Fullerenes, Graphene High strength, Conductivity, Lightweight Electronics, Drug delivery, Composite materials Nanoelectronics, Structural nanomaterials
Polymeric Nanoparticles PLGA, PEG-
based polymers Biodegradable, Biocompatible, Controlled release Drug delivery, Vaccine carriers Pharmaceutical nanotech
Lipid-Based Nanoparticles SLNs,
Liposomes, NLCs Biocompatibility, Targeted release Nutraceuticals, Cosmetics, Drug delivery Nano-pharmaceutical formulations
Quantum Dots CdSe, PbS (semiconductors) Size-tunable fluorescence, Optical stability Bioimaging, Solar cells, Diagnostics Nano-optics, Nanoimaging
Dendrimers Synthetic branched polymers High functionality, Nanoscale size Targeted drug/gene delivery, Imaging Biomedical nanotechnology
Ceramic Nanoparticles Silica, Alumina High stability, Porosity Bone regeneration, Catalysis Biomedical and catalytic nanotech
Magnetic Nanoparticles Fe_3O_4 , $CoFe_2O_4$ Magnetic responsiveness, Biocompatibility MRI, Targeted therapy, Data storage Magnetic nanotechnology, Medical
nanodevices
High-Entropy Alloy Nanoparticle Multi-metallic systems Thermal stability, Catalytic synergy Catalysis, Energy storage Advanced materials nanotechnology
Table no 1.2: Overview of Nanoparticle Types and Their Technological Relevance.

Conventional synthesis methods for ZnO nanoparticles, including sol-gel processing, hydrothermal techniques, precipitation methods, and physical approaches like vapor deposition. are effective in the producing all nanoparticles with controlled size and morphology. However,

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these methods were often involve into the use of hazardous precursors, high temperatures or pressures, and complex laboratory setups. These limitations not only elevate production costs but also pose environmental and health risks due to the generation of toxic by-products and non-biodegradable residues.

A wide spectrum of biological organisms has emerged as key agents in the eco-friendly synthesis of nanoparticles, playing an important role in the advancement of green nanotechnology (16). Among these, six primary groups—plants, algae, bacteria, fungi, virus, and yeast—are widely used due to their natural ability to reduce metal ions into stable nanoparticles without the need for toxic chemicals. Each of these organisms contributes unique bioactive molecules, such as proteins, polysaccharides, enzymes, phenolic compounds, flavonoids, and alkaloids,(17) which actively participate in the reduction, stabilization, and capping of nanoparticles. For instance, plant extracts are rich in secondary metabolites that not only speed up the reaction but also allow for size and shape control of the nanoparticles. Bacteria and fungi are also highly efficient producers due to their fast growth rates and the ease with which they can be genetically manipulated or cultured under laboratory conditions (18). Viruses, particularly virus-like particles (VLPs), it's engineered for viral nanoparticles, have gained significant attention for their ability to self-assemble into uniform nanostructures and are increasingly explored for the production of nanoparticles with distinct physicochemical properties.(19) Algae and yeast, although less frequently used, offer additional benefits such as high metal tolerance, scalability, and the ability to operate in varied environmental conditions. The involvement of these biological organisms enables a cleaner, more sustainable approach compared to traditional physical and chemical synthesis methods,(20) which often involve harsh conditions, toxic reagents, and high energy input. The nanoparticles synthesized using biological agents are generally biocompatible, it's making them highly suitable for use in biomedical applications including targeted drug delivery, wound healing, antimicrobial treatments, imaging, and diagnostics. Ongoing research continues to explore and optimize the roles of these organisms, aiming to improve yield,(21) control particle properties more precisely, and expand their application scope across industries beyond medicine, such as agriculture, food preservation, and environmental remediation.

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Fig 1.2: Nanoparticle synthesis by 6 primary groups of organism (plants, algae, bacteria, fungi, virus, and yeast)

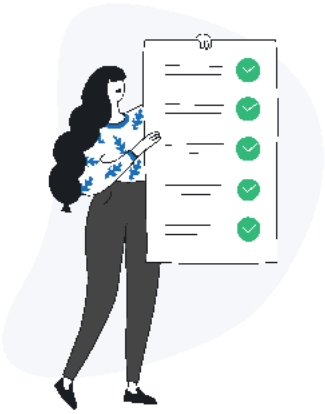
Green synthesis has gained popularity as an effective and sustainable solution to the limitations of conventional ZnO nanoparticle production.(22) This eco-friendly approach utilizes the reducing and stabilizing capabilities (23) of biological entities such as bacteria, fungi, algae, and plant extracts to synthesize ZnO nanoparticles under mild conditions.(24) Among these biological sources, plant-mediated synthesis stands out due to its simplicity, cost-effectiveness, scalability, and the abundance of phytochemicals in plants that serve as natural reducing and capping agents.(25) This method is not only removed the need for toxic chemicals but also aligns with environmentally conscious manufacturing practices.(26)

Plant Name Scientific Name Types of Nanoparticles Synthesized Key Bioactive Compounds

Neem Azadirachta indica Silver (Ag), Gold (Au), Zinc (Zn) etc. Flavonoids, terpenoids, tannins and azadirachtin

Tulsi (Holy Basil) Ocimum sanctum Silver (Ag), Gold (Au) Eugenol, flavonoids, tannins, alkaloids

Green Tea Camellia sinensis Silver (Ag), Gold (Au) Polyphenols (catechins), flavonoids



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Aloe vera Aloe barbadensis miller Silver (Ag), Iron Oxide (Fe_2O_3) Anthraquinones, flavonoids, carbohydrates, saponins
Moringa Moringa oleifera Silver (Ag) Ascorbic acid, flavonoids, phenolics
Eucalyptus Eucalyptus spp. Silver (Ag), Iron Oxide (Fe_2O_3) Tannins, flavonoids, terpenoids
Turmeric Curcuma longa Gold (Au) Curcumin, flavonoids, phenolics
Ginger Zingiber officinale Silver (Ag), Zinc (Zn) Gingerol, shogaol, flavonoids
Pteris Pteris vittata Zinc (Zn), Silver (Ag) Flavonoids, phenolic acids, terpenoids

Table no 1.3: The Natural Green Alchemists: The Synthesis of Nanoparticles by Plants:
Numerous plants, including neem, tulsi, green tea, aloe vera, and turmeric, (27) can naturally produce nanoparticles. Their extracts contain bioactive compounds that facilitate the conversion of metal salts into nanoparticles of zinc, gold, and silver. This method is widely recognized as "plant-based" or "green" synthesis. (28)
Pteris vittata, commonly known as the Chinese brake fern, is one such plant that has shown significant potential in this field.(29) Belonging to the Pteridaceae family, Pteris vittata is a well-known hyperaccumulator of heavy metals like arsenic and is often used in phytoremediation. Recent studies have also highlighted its ability to act as a bio-factory for the green synthesis of zinc oxide (ZnO) nanoparticles. (30) This ability is attributed to its rich phytochemical composition, which includes flavonoids, phenolic acids, terpenoids, and other secondary metabolites known for their strong reducing and stabilizing capabilities.(31)
The use of Pteris vittata in nanoparticle synthesis offers multiple advantages, including eco-friendliness, cost-effectiveness, and scalability. ZnO nanoparticles derived from Pteris extracts have demonstrated promising antimicrobial and antioxidant activities, making them suitable for various biomedical applications.(32) These include antimicrobial coatings, wound-healing materials, drug delivery systems, and environmental sensors. The renewable nature, fast growth, and widespread availability of Pteris vittata further enhance its appeal as a sustainable resource for nanoparticle production. (33)

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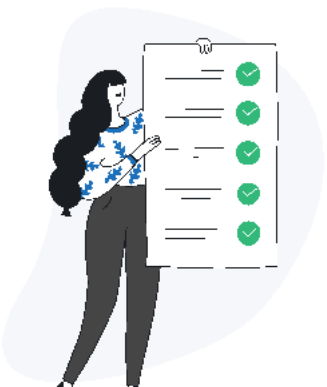
Chapter: 2
Review of Literature
2.1. Nanoparticle synthesis approaches:
2.1.1. Overview of Nanoparticle Synthesis Strategies: In the past few years, diverse techniques have been explored for synthesizing nanoparticles, including physical, chemical, and biological methods. (34) These techniques broadly fall under two main strategies: the top-down approach, which is physical in nature, and the bottom-up approach, it's includes the both chemical and biological methods. The top-down approach includes the breaking down all bulk materials into nanoscale structures through techniques like thermal decomposition, irradiation, diffusion, and arc discharge. (35) In contrast, the bottom-up approach builds nanoparticles molecules by molecules through techniques such as chemical reduction, electrochemical synthesis, polyol process, seeded growth, and biosynthesis .(36)

Fig 2.1: Difference between Top-down and Bottom down approach

2.1.2. Top-Down vs. Bottom-Up Approaches: They are both of chemical and physical synthesis methods are well-established, they often come with significant



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drawbacks.(37) These include the use of toxic reducing agents (e.g., sodium borohydride), hazardous solvents, exposure to UV and gamma radiation, and high energy requirements—all of which pose environmental and health risks. Due to these limitations, researchers are increasingly interested in developing eco-friendly alternatives for nanoparticle production. (38)

2.1.3. Limitations of Conventional Methods In between two synthesis biological synthesis methods have gained notable attention. These are involves into the use of biological systems—such as microorganisms and plants—to synthesize nanoparticles. This approach, often categorized under nanobiotechnology, aligns with natural biochemical processes, offering a cleaner, safer, and more sustainable route to nanoparticle production (39). Nanobiotechnology essentially utilizes biological molecules and systems for designing and producing nanoscale materials, drawing inspiration from natural nano-machinery observed in living cells.

2.1.4. Emergence of Biological Synthesis (Nanobiotechnology): Nature itself has provided compelling examples of biosynthesis. For example, magnetotactic bacteria are produce magnetite particles (40;41), diatoms form silica-based structures, and S-layer bacteria generate gypsum and calcium deposits. These act of nature have inspired the use of microorganisms including bacteria, actinomycetes, fungi, and algae—for nanoparticle synthesis (42). Additionally, many variety of plants extracts from leaves, flowers, bark, seeds, and peels all have also been used for green synthesis of nanoparticles (43).

2.1.5. Microbial and Plant-Based Nanoparticle Synthesis Biological synthesis can occur via two pathways: intracellular and extracellular. In intracellular synthesis, nanoparticles form within the cells (44), while in extracellular synthesis, secreted enzymes and biomolecules facilitate nanoparticle formation outside the cell (45).

2.1.6. Role of Pteris Plant in Nanoparticle Synthesis: In between various biological agents, the Pteris plants has appeared as a promising candidate for nanoparticle synthesis. This plant is known for its remarkable ability to uptake and accumulate heavy metals from the environment, a trait that plays a vital role in nanoparticle formation.(46) The green synthesis approach using Pteris offers several

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advantages, including low cost, environmental friendliness, ease of handling, and potential for large-scale application. These properties are make Pteris as a valuable and useful botanical resource for sustainable nanoparticle production.(47) Recent studies have highlighted the efficiency of Pteris vittata and related species in synthesizing metal nanoparticles, reinforcing their potential in nanobiotechnology and environmental remediation (48). Thus, the use of Pteris plants offers a natural, eco-conscious alternative to traditional chemical and physical synthesis methods.

2.2. Chemical Synthesis vs. Biosynthesis of Nanoparticles: Nanoparticle synthesis has traditionally relied on chemical methods, which often involve harsh reaction conditions, toxic reducing agents, and the generation of hazardous byproducts. These approaches, while offering precise control over particle size and morphology, raise environmental and biocompatibility concerns (49). In contrast, biosynthesis, which utilizes biological entities such as plants, bacteria, fungi, and viruses, has emerged as a greener and more sustainable alternative. This method not only reduces the use of harmful chemicals but also leverages the natural reducing and capping abilities of biological systems to produce the nanoparticles with better stability and functional properties (50). Moreover, biosynthesized nanoparticles often exhibit improved biocompatibility which making them particularly attention for biomedical applications.

2.3. Used of plants in ZnO-NPs Synthesis: A growing body of research highlights the successful green synthesis of the zinc oxide nanoparticles (ZnO-NPs) by using various plant species. According to a comprehensive study by Sharma et al. (51), more than 80 plant species across diverse families have been utilized for ZnO-NPs biosynthesis.(52) These include families such as Asteraceae, Lamiaceae, Zingiberaceae, and Meliaceae. Different parts of the plant like leaves, roots, bark, flowers, fruits, and peels—contain distinct phytochemicals that serve as reducing and capping agents during nanoparticle formation.(53)

The study emphasized the efficient synthesis of ZnO-NPs using extracts from:

• Aloe vera (Asphodelaceae):It's rich in saponins and flavonoids that enhance nanoparticle stability

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• Azadirachta indica (Meliaceae): It's Provides terpenoids and polyphenols aiding in nanoparticle reduction
• Camellia sinensis (Theaceae): Contains catechins and flavonoids with strong antioxidant properties
• Curcuma longa (Zingiberaceae): Curcumin in turmeric plays a useful role in the nanoparticle stabilization
• Ocimum sanctum (Lamiaceae): It's known for its high content of eugenol, flavonoids, and tannins
• Zingiber officinale (Zingiberaceae): Synthesized ZnO-NPs with gingerol and shogaol acting as bioreducers
• Moringa oleifera (Moringaceae): Ascorbic acid and phenolics in leaves facilitate nanoparticle synthesis
• Punica granatum (Lythraceae): Peel extracts; it's contain tannins and phenolic acids beneficial for ZnO-NPs formation
• Tridax procumbens (Asteraceae): Leaf extract serves as a potent bio-reducing medium
• Pteris vittata (Pteridaceae): It's a metal-hyperaccumulating fern which was recently explored for ZnO-NPs biosynthesis due to its rich flavonoid and phenolic content
These examples have the immense potentials of the plant biodiversity in the eco-friendly synthesis of zinc oxide nanoparticles. As research expands, more plant species are being investigated, not only for their synthesis capability but also for the enhanced antimicrobial, antioxidant, and catalytic activities imparted by the nanoparticles they help produce.

2.4. Overview of Green Synthesis of ZnO Nanoparticles Using Pteris Extract: The green synthesis of zinc oxide nanoparticles (ZnO-NPs) using Pteris plant extract represents a promising advancement in eco-friendly nanotechnology. This method utilizes the rich phytochemical profile of the Pteris genus—such as flavonoids,

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terpenoids, and phenolic compounds—they are act like as a reducing as well as stabilizing agents in the nanoparticle synthesis process. The approach removes the need for toxic chemicals commonly used in conventional methods, thereby reducing environmental risks and making the process more biocompatible. Studies have shown that Pteris-mediated ZnO-NPs exhibit notable antimicrobial and antioxidant activities, attributed to the synergistic effect of the nanoparticle surface chemistry and bioactive phytoconstituents derived from the extract. These features make them highly suitable for biomedical and environmental applications.(54,55).

2.5. Phytochemical Composition of Pteris Extract: The Pteris genus, a group of ferns known for their hyperaccumulating ability and medicinal use (56), contains a variety of bioactive compounds that contribute to the stabilization and production of Zinc oxide nanoparticles:

• Flavonoids: These are potent antioxidants with reducing properties that facilitate the transformation of zinc ions (Zn^{2+}) into zinc oxide nanoparticles.(57) They also play a key role in stabilizing the nanoparticles and controlling their morphology.(58)

• Phenolic compounds: Known for their strong electron-donating capacity, phenolics contribute to the reduction of zinc salts and enhance the overall stability and bioactivity of the ZnO-NPs.(59)

• Terpenoids and saponins: These act as natural surfactants, helping to regulate both the size and prevent aggregation of nanoparticles, thereby maintaining their functional integrity.

The use of Pteris extract in ZnO-NPs synthesis not only promotes an eco-friendly route of nanoparticle production but also imparts biofunctional properties that can be harnessed in biomedical, agricultural, and environmental applications. This plant-mediated method offers simplicity, cost-effectiveness, and reduced toxicity—making it a viable alternative to conventional chemical synthesis techniques. (60)

2.6. Phytochemical Profiling of Pteris Species for Green Synthesis of Zinc Oxide Nanoparticles: The effectiveness of Pteris species in the green synthesis of zinc oxide nanoparticles (ZnO-NPs) is attributed to their diverse and rich phytochemical composition. (61)These naturally occurring compounds not only enable the eco-

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friendly reduction of metal ions but it's also influence the characteristics of the manufactured nanoparticles.(62)

Pteris plant extract contains many bioactive constituents, which are including:

• Flavonoids and phenolic acids – It's known for their strong antioxidant and electron-donating capabilities

• Tannins – which provide reducing and stabilizing action

• Terpenoids and glycosides – It's important for the shape and size control during nanoparticle formation

• Saponins – function as natural surfactants and stabilizing agents

• Alkaloids and steroids – which may aid in particle nucleation and antimicrobial properties

• Polysaccharides and proteins – acting as biocompatible capping agents to prevent aggregation

• Essential minerals – like zn2+, calcium, and iron etc.

In the green synthesis process, flavonoids and phenolic compounds donate electrons to reduce Zn^{2+} ions into ZnO nanoparticles,(63) initiating nucleation. Once nucleation begins, saponins, proteins, and polysaccharides act as a natural capping and stabilizing reagent, forming a biocompatible shell around the nanoparticles. (64) This prevents agglomeration and enhances nanoparticle dispersion in aqueous media.

Additionally, terpenoids and glycosides are useful in controlling the shape and size of the ZnO-NPs, ensuring a uniform morphology, which is crucial for biomedical and industrial applications.(65) These phytochemicals collectively make Pteris extract a highly efficient and eco-friendly medium for the synthesis of ZnO nanoparticles.(66)

Beyond synthesis, these compounds are also help to increase the biological activity of the resulting nanoparticles.(67) The antimicrobial, antioxidant, and wound-healing properties of ZnO-NPs synthesized by using Pteris extract are notably improved, making them suitable for biomedical, environmental, and cosmetic applications. (68)

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2.7. Mechanism of Synthesis: The synthesis of the zinc oxide nanoparticles (ZnO-NPs) by using the Pteris plant extract (69), the process involves the following key steps:

• Reduction of Zinc Ions: Zinc ions (Zn^{2+}) are reduced and transformed into zinc oxide nanoparticles (ZnO-NPs) by the electron-donating action of phytochemicals present in Pteris extract, (70) such as flavonoids and phenolic compounds. (71) This initiates the nucleation process essential for nanoparticle formation.

Zn2++2OH- → heat → ZnO+ H2O

• Stabilization and Capping of ZnO-NPs: Bioactive compounds like saponins, proteins, and polysaccharides in the Pteris extract act as natural stabilizing and capping agents. These substances form a protective,(72) biocompatible shell around the nanoparticles, which prevents agglomeration and enhances stability.(73)

• Shape and Size Control: Terpenoids and glycosides in the Pteris extract further assist for regulating the morphology (shape and size) of the synthesized ZnO-NPs, resulting in nanoparticles with desirable properties for it's biomedical and antimicrobial applications.(74)

2.8. Advantages of Pteris Plant for Green Synthesis of ZnO Nanoparticles:

a) Eco-Friendly and Sustainable Technique: The green synthesis of ZnO nanoparticles using Pteris extract avoids the use of hazardous chemicals and energy-intensive methods.(75) This environmentally benign process supports sustainable nanotechnology and stands with the several UN Sustainable Development Goals (SDGs).

b) Economic Sustainability: Pteris plants are naturally abundant, easy to cultivate, and require minimal processing. This makes them a cost-effective and scalable option for large-scale ZnO-NPs production, especially in resource-limited settings.(76)

c) Biocompatibility: ZnO nanoparticles synthesized using Pteris extract are biocompatible and free from toxic residues. This makes them ideal for biomedical and pharmaceutical applications, including antibacterial formulations, drug delivery, and tissue engineering.(77)

d) Improved Stability: The natural proteins, polysaccharides, and saponins in Pteris extract act as capping agents that prevent agglomeration. This gives impact on high colloidal stability and increases the shelf life of the nanoparticles.(78)

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e) Controllable Physical Properties: Parameters like extract concentration, pH, reaction time, and temperature can be varied to effectively control the size, shape, and surface morphology of ZnO-NPs. The diverse phytochemical composition of Pteris aids in tunable synthesis for specific functional applications.(79)

f) Antioxidant and Antimicrobial Properties: Natural flavonoids and phenolics in Pteris provide antioxidant protection during nanoparticle synthesis. These properties not only stabilize ZnO-NPs but also enhance their antimicrobial efficacy, making them valuable for health care and environmental applications.(80)

2.9. Role of Pteris Plant in the Synthesis of Nanoparticles: Among the many plant sources explored for green synthesis, (81) the Pteris plant (a fern belonging to the Pteridaceae family) has surfaced as a promising candidate for it's eco-friendly nanoparticles production. The extract of the Pteris leaves is a rich in bio-active phytochemicals such as flavonoids, phenolic-compounds, saponins, glycosides, terpenoids, alkaloids, and poly-saccharides.(82) These compounds serve both roles in the synthesis of ZnO nanoparticles:

- As reducing agents, flavonoids and phenolics donate electrons to convert Zn^{2+} ions into ZnO nanoparticles.(83)
- As stabilizing and capping agents, polysaccharides, proteins, and saponins coat the nanoparticles, preventing agglomeration and ensuring uniform shape and dispersion.(84)

The use of Pteris in nanoparticle synthesis offers multiple advantages:

- Non-toxic and biodegradable synthesis method
- Dual functionality,behave as reducing as well as stabilizing reagent.
- Sufficient and renewable natural resource, provide them medicinal and ecological significance
- Biocompatibility, it's making suitable for biomedical and environmental applications.

A study by demonstrated (85) the efficient formation of ZnO nanoparticles using Pteris vittata extract under mild conditions, producing uniformly dispersed particles with potent antimicrobial

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activity. This confirms the viability and effectiveness of Pteris-based green synthesis in modern nanotechnology.(86)

2.10. Applications of Pteris-Mediated Zinc Oxide Nanoparticles: Zinc oxide nanoparticles synthesized via Pteris plant extract exhibit a wide range of applications due to their excellent physicochemical stability, biocompatibility, and strong biological activity, especially their antibacterial, antifungal, antioxidant, and UV-protective properties. These characteristics makes them Pteris-mediated ZnO-NPs highly valuable in medicine, environmental protection, and agriculture.

2.10.1. Biomedical Applications: Pteris-based ZnO-NPs are especially promising into the pharmaceutical and healthcare industries due to their better biological properties given by the phytochemicals involved in their green synthesis process.

a) Wound Healing and Antiseptic Treatments: ZnO-NPs synthesized from Pteris extract possess potent antimicrobial and anti-inflammatory properties, making them ideal for topical applications in burns, ulcers, and wounds. The flavonoids and phenolics from Pteris help in tissue repair and healing.

- Promote fibroblast proliferation and collagen formation
- Support re-epithelialization and angiogenesis
- Inhibit microbial colonization and reduce wound infections

These characteristics make them effective ingredients in ointments, hydrogels, sprays, and wound dressings.

b) Antibacterial and Antifungal Therapy: ZnO-NPs have demonstrated effectiveness against many types of antibiotic-resistant bacterial strains, such as Staphylococcus aureus, and fungi like Candida albicans. Pteris-derived ZnO-NPs can:

- Penetrate microbial membranes and cause oxidative damage
- Interfere with DNA replication and protein synthesis
- Disrupt biofilms, crucial in chronic infections

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This makes them suitable for coatings on surgical instruments, implants, and catheters.

c) Targeted Drug Delivery: Thanks to their nano-size, high surface-to-volume ratio, and surface functional groups, Pteris-mediated ZnO-NPs can be tailored for drug loading and release, including:

- Anticancer drugs (e.g., doxorubicin)
- Anti-inflammatory agents
- Antibiotics (e.g., ciprofloxacin)

These features improve the targeted delivery, enhancing treatment effectiveness while reducing side effects.

d) Cancer Therapy: Preliminary in vitro studies suggest that Pteris-synthesized ZnO-NPs can induce oxidative stress and apoptosis in cancer cells such as MCF-7 (breast cancer) and HeLa (cervical cancer).

- Exhibit selective cytotoxicity toward cancer cells
- Minimize damage to healthy tissues
- Offer potential for photodynamic and chemotherapeutic applications (87)

2.10.2. Environmental Applications: Pteris-mediated ZnO-NPs are effective agents in environmental remediation, particularly in wastewater treatment and antimicrobial surface applications.

a) Water Disinfection and Filtration: ZnO-NPs can be embedded in filtration membranes or coating materials to disinfect drinking water by eliminating pathogens.

- Kill bacteria, viruses, and fungi
- Prevent biofilm formation in filters
- Release minimal toxic byproducts, making them safer than conventional disinfectants

b) Antimicrobial Surface Coatings: These ZnO-NPs can be used on hospital surfaces, masks, gloves, and high-touch surfaces (like door handles and elevator buttons) to reduce pathogen transmission.

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• Provide long-lasting antimicrobial protection.
• Reduce environmental toxicity as compared to the chemically synthesized ZnO-NPs (88)
2.10.3. Agricultural Applications: ZnONPs derived from Pteris extracts are an eco-friendly alternative to synthetic agrochemicals, offering antibacterial, antifungal, and plant-growth-promoting properties.
a) Control of Plant Pathogens: ZnO-NPs can control diseases caused by plant pathogens such as:
• Ralstonia solanacearum (bacterial wilt)
• Alternaria spp. (leaf spot)
• Fusarium oxysporum (root rot)
They act by disrupting cell walls, metabolic processes, and genetic material of phytopathogens, serving as a biopesticide.
b) Seed Germination and Plant Growth: Low concentrations of ZnO-NPs improve:
• Seed germination rates
• Root elongation and shoot development
• Nutrient absorption and plant biomass
However, the effect is dose-dependent; high concentrations may be phytotoxic.
c) Food Packaging and Preservation: The antimicrobial properties of ZnO-NPs make them ideal for active food packaging. They can be incorporated into:
• Biodegradable films (e.g., chitosan or starch-based)
• Edible coatings for fruits and vegetables
• Antimicrobial wrapping materials
These applications help prevent spoilage by organisms like Listeria monocytogenes and Salmonella enterica, thus enhancing shelf life and reducing food waste.(89)
2.11. Pteris-Mediated Formation of Zinc Oxide Nanoparticles (ZnO-NPs): The physicochemical properties of zinc oxide nanoparticles (ZnO-NPs)—such as their

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morphology, particle size, surface area, crystallinity, and dispersion—significantly influence their optical, antibacterial, and catalytic activities. When synthesized using Pteris extracts, ZnO NPs typically exhibit the following features:

• They often possess hexagonal wurtzite crystal structures and exhibit spherical, rod-like, or irregular morphologies, depending on the phytochemical composition of the extract and synthesis parameters.

• Their particle sizes generally range from 20 to 100 nanometers, though finer control during synthesis can produce smaller sizes.

• The nanoparticles may show good dispersion or slight agglomeration, influenced by the concentration of biomolecules (e.g., polyphenols, flavonoids, proteins) in Pteris extract, which act as both reducing and stabilizing agents.

To confirm the synthesis and analyze the characteristics of Pteris-mediated ZnO NPs, several analytical techniques are employed:

• UV-Visible Spectroscopy: Shows a characteristic absorption peak in the range of 350–380 nm, attributed to the excitonic transitions of ZnO and its surface plasmon resonance-like behavior.

• Fourier-Transform Infrared Spectroscopy (FTIR): Identifies the functional groups (e.g., $\equiv$OH, $\equiv$COOH, NH_2) from the plant extract involved in the reduction of Zn^{2+} ions and the stabilization of the ZnO NPs.

• Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM): Reveal the size, shape, and morphology of the nanoparticles.

• X-ray Diffraction (XRD): Confirms the crystalline nature and phase purity of the ZnO nanoparticles, typically showing diffraction peaks matching the wurtzite structure.

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The combination of these techniques confirms that Pteris-derived phytochemicals successfully mediate the green synthesis of ZnO NPs, offering an eco-friendly and cost-effective alternative to chemical synthesis routes.(90)

2.12. Mechanisms of Antibacterial Activity of Zinc Oxide Nanoparticles Synthesized Using Pteris: Zinc oxide nanoparticles (ZnO-NPs) created using Pteris plant extracts have shown strong potential as antimicrobial agents. These green-synthesized nanoparticles are effective against a wide range of harmful microbes, including:

- Gram-positive bacteria like Staphylococcus aureus & Bacillus subtilis
- Gram-negavites bacteria like Pseudomonas aeruginosa and E.coli
- Fungal pathogens like Candida albicans
- Even strains of bacteria are also become resistant to many types of antibiotics

The powerful antibacterial action of these ZnO-NPs is believed to work through a combination of mechanisms:

a) Breaking Down Bacterial Membranes: ZnO-NPs stick to the surface of bacteria, disturbing their protective outer layers. It's the reason of the cell membranes to become leaky and leading to the loss of vital cell contents and finally cells becomes death.

b) Creating Harmful Reactive Oxygen Species (ROS): These nanoparticles can trigger the formation of reactive molecules such as superoxides and hydroxyl radicals, which attack and damage the internal structures of microbes—including fats, proteins, and DNA.

c) Releasing Zinc Ions (Zn^{2+}): When ZnO-NPs release zinc ions, these ions can enter microbial cells and interfere with important enzyme activities and metabolic functions, further weakening or killing the bacteria.

d) Disrupting DNA and Protein Synthesis: By damaging genetic material and essential proteins, ZnO-NPs prevent bacteria from growing and reproducing, effectively halting infections from spreading.

Studies have shown that ZnO-NPs synthesized using Pteris extracts may have enhanced antibacterial effects due to the presence of natural bioactive compounds in the plant. These

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compounds can either add their own antimicrobial effects or help the nanoparticles bind more effectively to microbes, making the treatment more efficient.

2.13. The Impact of Synthesis Parameters: The efficiency, stability, size, and biological effectiveness of zinc oxide nanoparticles (ZnO-NPs) synthesized using Pteris extracts are highly influenced by various synthesis conditions. These parameters directly affect the nanoparticles physicochemical properties and their subsequent applications. Key factors include:

2.13.1. Zinc Salt Concentration: The amount of zinc precursor (such as zinc acetate or zinc nitrate or zinc sulphate) used during synthesis plays a critical role. Higher concentrations can lead to the formation of larger and more aggregated ZnO particles, which may reduce their surface activity and antibacterial effectiveness.

2.13.2. Pteris Plant Extract Volume: The concentration of Pteris extract determines the availability of phytochemicals that act as reducing and stabilizing agents. An optimal plant-to-metal salt ratio is essential for efficient synthesis, proper particle size control, and improved dispersion stability.

2.13.3. pH of the Reaction Medium: The pH level of the synthesis solution help to increases the nanoformation. Alkaline conditions (usually pH 8 12) tend to promote the creation of smaller, well-dispersed ZnO-NPs with enhanced crystallinity and better antibacterial performance, while acidic conditions may lead to incomplete reactions or irregular shapes.

2.13.4. Temperature and Reaction Duration: Elevated temperatures enhance the rate of chemical reductions and nucleation, leading to the faster synthesis and more uniform particle formation. Reaction time also plays a crucial role—insufficient time may result in incomplete nanoparticle formation, while excessive time can cause unwanted aggregation. (91)

2.14. Uses and Comparative Efficiency: Comparative analyses indicate that zinc oxide nanoparticles (ZnO-NPs) synthesized using Pteris extracts frequently demonstrate improved biocompatibility, antimicrobial performance, and environmental friendliness compared to their chemically synthesized counterparts. Their eco-

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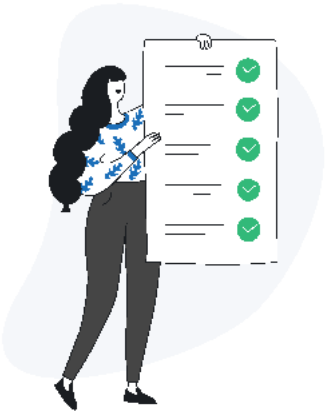
friendly origin and reduced cytotoxicity make them suitable for a broad range of practical applications, including:

- Antibacterial coatings is for the wound dressings and the surgical tools
- Disinfection agents in water purification systems
- Topical antimicrobial is apply for skin infections and inflammation
- Slow-release platforms for drug delivery, especially for antibiotics and anti-inflammatory drugs
- Biofertilizers or nano-pesticides in sustainable agriculture

Research evidence, including recent in vivo and in vitro studies, suggests that Pteris-based ZnO-NPs can accelerate wound healing and suppress microbial growth more effectively due to the synergistic interaction between ZnO and the plant's bioactive compounds. Their capability to produce reactive oxygen species (ROS) and disrupt microbial membranes enhances their effectiveness even against resistant strains.(92)



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Chapter: 3
Methods & Materials:
3.1 Objective:
Our main objective of the dissertation work is;
• Isolate and purify of nanoparticles from the pteris extract
• Characterization of nanoparticles by UV-Visible Spectrophotometry, Dynamic Light Scattering analyzer, XRD, and FTIR.
• Identify the antimicrobial activity of nanoparticles by Agar Diffusion method and Broth Dilution Assay.
3.2 Materials:
The chemicals Zinc sulphate (ZnSo4), Plant extract, Distilled water, NaOH, HCL, Nutrient broth (NB), Luria-Bertani broth (LB), Iodonitrotetrazolium chloride (INT dye), DMSO, KBr.
3.3 Sample collection:
Fresh plant material was collected from the South 24 Parganas district, specifically in and around the area near Dinabandhu Andrews College, located in Garia, Kolkata, West Bengal, India (Latitude: 22.4491° N, Longitude: 88.3915° E). The nearby region includes localities such as Boral, Baishnabghata, Narendrapur, and New Garia, which are known for their mix of urban and semi-natural green spaces. These areas host diverse plant species and offer suitable conditions for the natural growth of ferns and other flora.
The collection was performed manually during the daytime under suitable weather conditions. Healthy and mature plant specimens were carefully selected to ensure maximum phytochemical content and biological activity. Immediately after harvesting, the plant material was placed in sterile, labeled polythene bags and transported to the laboratory under ambient conditions to maintain freshness and prevent degradation. All necessary precautions were taken to avoid contamination during the collection and transport processes.

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Fig 3.1: Pteris plant

3.4 Sample Identification:

The collected plant specimen was taxonomically identified with the assistance of experienced faculty members from the Department of Botany at Dinabandhu Andrews College, Garia, Kolkata, West Bengal, India (Pin – 700084). The identification process involved careful examination of morphological features and comparison with standard botanical references and herbarium specimens. To ensure authenticity and reproducibility, the identified specimen was assigned a unique voucher number and preserved in the Herbarium of the Botany Department at Dinabandhu Andrews College. This voucher specimen will serve as a reference for future studies and verification.

3.5 Solution preparation:

To prepare a 0.1 M zinc sulphate ($ZnSO_4$) solution, accurately weigh 28.75 g of zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$, molar mass 287.54 g/mol) using a digital balance. Dissolve the weighed salt in approximately 800 mL of distilled water in a beaker or volumetric flask, stirring until it is completely dissolved. After the dissolving transfer it into the solution to a 1-liter volumetric flask if needed, and then add distilled water to make sure the final volume will be become exact 1 liter. Mix the solution thoroughly to ensure uniformity.(93)

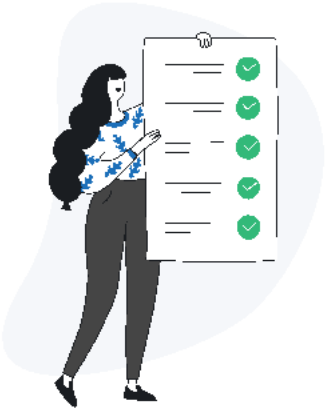

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Fig 3.2: ZnSo4 solution

3.6 Plant Extract Preparation:

Fresh Pteris leaves were collected and thoroughly rinsed to eliminate dust and other surface impurities. Initially, the leaves were washed with tap water to remove visible dirt, followed by rinsing with distilled water to eliminate any remaining surface contaminants. After cleaning, approximately 35 grams of the leaves were weighed using a digital balance. The cleaned leaves were then crushed using a mortar and pestle, with a small amount of distilled water added to facilitate the grinding process. The resulting paste was transferred into a beaker, and the aqueous mixture was gently heated on a magnetic stirrer hot plate at a temperature of approximately 70–80 °C for 30 minutes. This heating process facilitated the extraction of bioactive compounds from the plant material.

After the heat process, the mixture was then allowed to cool at the room temperature. It was then filtered through Whatman No.1 filter paper to remove any residual solid plant material. The clear filtrate obtained was collected and stored in amber-colored bottles to prevent any type of light-induced degradation of the phytochemicals. The extract was then store at 4 °C until further use.

This prepared Pteris leaf extract served as a natural reducing and stabilizing agent in the green synthesis of zinc oxide (ZnO) nanoparticles, following established procedures (94, 95).

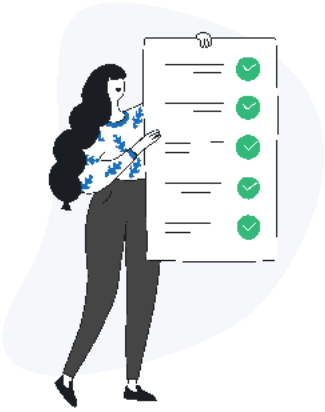

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Fig 3.3: pteris extract

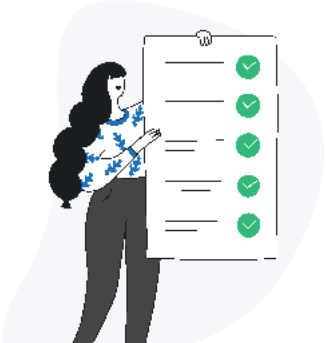
3.7. Green Synthesis of Zinc Oxide Nanoparticles (ZnO-NPs) formation method: The zinc oxide nanoparticles were synthesized by using a simple and eco-friendly green synthesis approach.

• At first, a 0.1 M solution of zinc sulfate ($ZnSO_4 \bullet 7H_2O$) was freshly prepared (mandetory) by dissolving the required amount of the salt in distilled water.

• To carry out the synthesis, 90 mL of this zinc sulfate solution was poured into a clean beaker, and 10 mL of the Pteris leaf extract was slowly added while continuously stirring the mixture with a magnetic stirrer. The entire process was conducted at a controlled temperature of around 60–80 °C for 1 to 2 hours. During this time, a gradual change in the color and clarity of the solution was observed, indicating the beginning of nanoparticle formation. (96)

• As the reaction progressed, a white precipitate started forming at the bottom of the beaker, confirming the synthesis of ZnO nanoparticles. Once the reaction was geting finished, the mixture was allowed to cool down to room temperature. The nanoparticles were then separated by centrifugation at 10,000 rpm for about 15 minutes. To ensure purity, the collected precipitate was washed several times with distilled water and ethanol, removing any leftover impurities or plant residues.

• Finally, the washed nanoparticles were dried in a hot air oven at 60 °C for 6–8 hours to obtain fine ZnO nanoparticle powder. The dried product was carefully stored in airtight containers for further testing and characterization.



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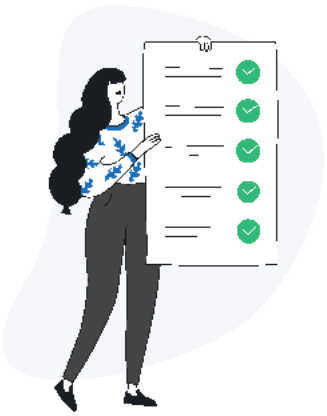
This green synthesis technique relies entirely on the natural phytochemicals present in the Pteris extract, which act as both reducing and stabilizing agents. It offers a sustainable and non-toxic alternative to traditional chemical methods and aligns well with environmentally friendly research practices. (97)

Fig 3.3: Complete process of Zn NPs synthesis

3.8 Characterization:

3.8.1 Characterization of Zinc Oxide Nanoparticles: To better understand the properties of the green-synthesized zinc oxide nanoparticles, a series of standard characterization techniques were carried out. UV–visible spectroscopy was first used to confirm the formation of nanoparticles by detecting their typical optical absorption pattern. (98) To identify the plant-based functional groups involved in reducing and stabilizing the nanoparticles, FTIR analysis was performed. The crystal structure and purity of the ZnO-NPs were examined by using X-ray diffraction method. Additionally, dynamic light



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scattering (DLS) was used to determine the average particle size and distribution in solution, offering insight into their stability in a colloidal state. These methods together provided a comprehensive understanding of the synthesized nanoparticles, following approaches similar to those described in earlier studies (99).

3.8.1.1 UV-Visible Spectroscopy of synthesized nanoparticles:

UV–Visible spectroscopy was utilized as a standard analytical technique to confirm the biosynthesis and evaluate the optical properties of zinc oxide nanoparticles (ZnO-NPs) synthesized using Pteris leaf extract. This non-destructive method is widely applied in nanoparticle research due to its effectiveness in monitoring the electronic transitions and surface plasmon resonance (SPR) behavior characteristic of metal and metal oxide nanoparticles. For spectral analysis, the synthesized ZnO-NPs colloidal solution was subjected to examination without dilution. The measurements were carried out using a calibrated double-beam UV–Vis spectrophotometer equipped with quartz cuvettes having a 1 cm path length. Before sample scanning, baseline correction was performed using distilled water to ensure accuracy. (100) The absorption spectra were recorded across a wavelength range of 200 to 800 nm at ambient room temperature. The scanning range was chosen based on established literature, as it encompasses the region where ZnO nanoparticles typically exhibit a pronounced absorbance peak corresponding to their band gap energy. Identification of this peak provides supportive evidence for nanoparticle formation and insights into the size and morphology of the synthesized material. The methodology adheres to widely accepted protocols for green nanoparticle characterization, as reported in previous studies involving plant-mediated ZnO-NPs synthesis . This approach ensures methodological consistency and scientific reliability in characterizing biogenically synthesized nanoparticles. (101)

3.8.1.2 Particle size measurement by dynamic light scattering (DLS) experiment:

Dynamic Light Scattering (DLS) was employed to assess the average hydrodynamic size and particle size distribution of the zinc oxide nanoparticles (ZnO-NPs) synthesized via the green route using Pteris leaf extract. This technique analyzes the Brownian motion of suspended nanoparticles and measures fluctuations in the intensity of scattered light to estimate particle size in a colloidal system. (102) The nanoparticle suspension was first filtered using a 0.22 µm membrane filter to eliminate any dust or large

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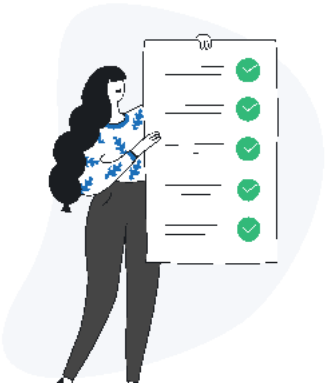
due to the nanoparticles alone. The plates were then incubated in an inverted position at 37 ± 1°C for 24 hours. After incubation, the plates were examined for clear zones around the wells, which indicate bacterial growth inhibition. (119). To make sure the perfect result, each test was conducted in triplicate.

Fig 3.5: Bacterial culture plates treated by ZnNPs solution; A) Escherichia coli and B) Bacillus subtilis
3.8.2.2. Broth micro dilution assay:
The antibacterial efficacy of the green-synthesized zinc oxide nanoparticles (ZnO-NPs) was further evaluated using a broth micro dilution method against Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative). Luria-Bertani (LB) broth was applied as the culture medium for this assay. (120) Each well of a sterile 96-well microtiter plate was set up to contain a total volume of 200 µL. For all treatments, 20 µL of fresh bacterial inoculum—standardized to match 0.5 McFarland turbidity—was used. The remaining 180 µL consisted of varying proportions of LB broth and ZnO-NPs solution, depending on the nanoparticle concentration being tested. The nanodose concentrations used were 0.25, 0.5, 0.75, 1.0, and 1.25 mg/mL . For the control sets, 180 µL of LB broth was mixed with the 20 µL of bacterial inoculum, with no nanoparticles



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Pteris leaf extract and to understand their role in the reduction and stabilization of zinc oxide nanoparticles (ZnO-NPs). This technique is especially useful for detecting specific chemical bonds and functional groups based on their characteristic absorption of infrared light. To begin the analysis, a small quantity of the synthesized and dried ZnO nanoparticle powder was mixed with spectroscopic-grade potassium bromide (KBr) in a 1:100 ratio by weight. This mixture was then finely ground using a clean mortar and pestle to ensure even blending. The resulting powder was then compressed into a transparent pellet by using a hydraulic press under high pressure. The FTIR spectra were recorded using an FTIR spectrometer in the range of 4000 to 400 cm^{-1} at room temperature. The background spectrum was measured using a pure KBr pellet, which was automatically subtracted from the sample spectra to eliminate interference. The main absorption peaks were carefully analyzed to determine the functional groups involved, particularly those from the plant extract that may have acted as capping and stabilizing agents during nanoparticle synthesis.(105)

Sl. No. FTIR spectral
region (cm-1) Assigned functional groups
1 749-500 R-C-H (106)
2 941-803 C–H in plane deformation with aromatic- ring stretching (107)
3 1090-1049 -C-O-C- (108)
4 1260-1100 Amide III/ N-H bending and the (C-N) stretching vibration (109)
5 1380-1330 CH3 deformation (110)
6 1490-1401 Symmetrical stretching vibrations of –COO (111)
7 1580-1510 Amide II/ N-H bending vibration (112)
8 1700-1600 Amide I/ C=O stretching vibration (113)
9 2973-2541 CH3/ C-H stretching vibration (114)
10 3450-3250 N-H stretching (115)
11 3600-3200 O-H stretching of alcohols or phenols (116)
Table 3.1:List of different FTIR spectral regions with their assigned functional groups.

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3.8.2 Antimicrobial Activity of Zinc nanoparticle: To assess the antibacterial potential of the biosynthesized ZnO nanoparticles, two widely accepted microbiological assays were conducted: the agar well diffusion methods and the broth dilution methods. These tests helped determine how effectively the nanoparticles inhibited bacterial growth. In the agar dilution method, different concentrations of ZnO nanoparticles were mixed into LB agar media, and bacterial cultures were spread on the surface. After incubation, any visible inhibition of bacterial growth was noted as a sign of antimicrobial activity. For a more quantitative analysis, the broth dilution method was employed using LB broth supplemented with p-iodonitrotetrazolium violet (INT) dye, which changes color in the presence of metabolically active bacteria. By visualized the dye's colour shift, the minimum inhibitory concentration (MIC) of the ZnO-NPs was determined—this being the lowest concentration capable of preventing visible bacterial population. These procedures followed standard protocols as described in earlier research on nanoparticle antimicrobial testing (117), and they provided a reliable measure of the synthesized ZnO-NPs' antibacterial effectiveness.

3.8.2.1 Agar well diffusion methods analysis:

To assess the antibacterial potential of the biosynthesized zinc oxide nanoparticles (ZnO-NPs), an agar well diffusion method was carried out using two model bacterial strains: Bacillus subtilis (Gram-positive) and Escherichia coli (Gram-negative). Nutrient agar served as the culture medium and was prepared according to the standard manufacturer's instructions. Once the medium was sterilized by autoclaving at 121°C for 15 minutes, it was poured into sterile Petri dishes and allowed to solidify in a clean environment. Fresh overnight bacterial cultures were grown in nutrient broth and adjusted to a turbidity equivalent to the 0.5 McFarland standard. By the help of sterile cotton swabs all cultures were then evenly spread into the surface of the solidified agar to make clear a consistent microbial lawn. (118) After inoculation, four wells (each 10 mm in diameter) were carefully made in the agar using a sterile cork borer. Three of the wells were filled with 50 µL of ZnO-NP solutions at concentrations of 25, 50, and 100 µg/mL, respectively. The remaining well were acted as a negative control and was filled with 50 µL of non-ionized distilled water to verify that any antibacterial activity observed was

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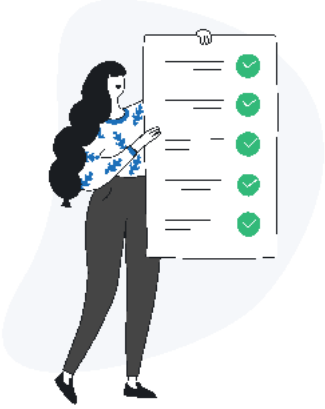
due to the nanoparticles alone. The plates were then incubated in an inverted position at 37 ± 1°C for 24 hours. After incubation, the plates were examined for clear zones around the wells, which indicate bacterial growth inhibition. (119). To make the perfect result, each test was conducted in triplicate.

Fig 3.4: Bacterial culture plates treated by ZnNPs solution; A) Escherichia coli and B) Bacillus subtilis

3.8.2.2. Broth dilution assay:

The antibacterial efficacy of the green-synthesized zinc oxide nanoparticles (ZnO-NPs) was further evaluated using a broth dilution method against Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative). Luria-Bertani (LB) broth was applied as the culture medium for this assay. (120) Each well of a sterile 96-well microtiter plate was set up to contain a total volume of 200 µL. For all treatments, 20 µL of fresh bacterial inoculum—standardized to match 0.5 McFarland turbidity—was used. The remaining 180 µL consisted of varying proportions of LB broth and ZnO-NPs solution, depending on the nanoparticle concentration being tested. The nanodose concentrations used were 0.25, 0.5, 0.75, 1.0, and 1.25 mg/mL . For the control sets, 180 µL of LB broth was mixed with 20 µL of bacterial inoculum, with no nanoparticles



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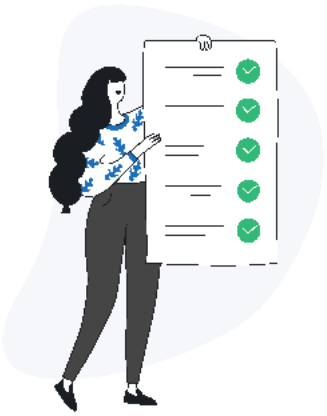
added. For treated wells, the nanoparticle solution was adjusted to the desired concentration, and the volume of LB broth was reduced accordingly to maintain a constant total volume of 200 µL. The plates were incubated overnight at 37 ± 1°C. Following incubation, 20 µL of an INT (iodonitrotetrazolium) dye solution was added to each well as a colorimetric indicator of bacterial viability. The plates were then kept in the dark for an additional 30 minutes to allow proper dye reaction. Any color change was observed visually to assess microbial growth inhibition. All treatments were performed in triplicate to ensure consistency and reproducibility. (121).

Fig 3.5: bacterial culture treatment under 96- well dilution plate ; A) Overnight incubated plate, B) 30 mins dark incubated plate after apply INT dye.



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Chapter: 4
Results and discussions
4.1. In vitro Green Synthesis of Zinc Nanoparticles:
Zinc nanoparticles were synthesized in vitro using an eco-friendly approach by exposing Pteris extract to a 0.1 M aqueous solution of zinc sulphate ($ZnSO_4$). Upon the addition of the plant extract to the metal salt solution, a time-dependent color change was observed from dark green to a light yellowish-green hue. This color shift indicated the successful biosynthesis of zinc nanoparticles (ZnO-NPs), suggesting the reduction of Zn^{2+} ions and the formation of stable nanoparticles. The visual change in the reaction mixture is attributed to the excitation of surface plasmon resonance (SPR), which typically accompanies the formation of metal nanoparticles.
No color change was observed in either the zinc sulfate solution (negative control) or the Pteris extract alone (positive control), confirming that the color shift was due to nanoparticle synthesis and not due to any inherent property of the individual components.
Similar biosynthetic approaches have been reported earlier using different biological sources. For instance, Ramesh et al. (2014)(122) reported a comparable green synthesis of ZnO-NPs utilizing Calotropis gigantea leaf extract, where a noticeable color change indicated the formation of nanoparticles. Likewise, Vijayaraghavan et al. (2012)(123) successfully employed Aspergillus flavus for the biosynthesis of zinc oxide nanoparticles, observing similar visual cues during nanoparticle formation. In another study, Bhuyan et al. (2015)(124) demonstrated the synthesis of ZnO-NPs using Azadirachta indica leaf extract, reinforcing the efficacy of plant-based systems for nanoparticle synthesis.
These findings align with our current observation that plant extracts, due to their rich phytochemical composition (including flavonoids, terpenoids, and polyphenols), act as both reducing and stabilizing agents in nanoparticle synthesis. The simplicity, cost-effectiveness, and environmental compatibility of this method position it as a promising alternative to traditional chemical synthesis methods.

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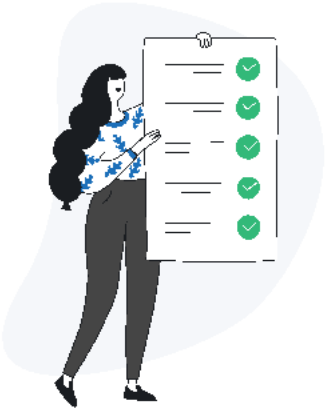
Fig: 4.1 Visual change in colour of the reaction mixture compared with the control set; A) Zinc sulfate solution; B) reaction mixture; C) Pteris extract.

4.2. Characterization of the Zing nano particles:

4.2.1. UV-Visible Spectrophotometric Analysis of Zinc Nanoparticles Synthesized Using Pteris Extract:

To confirm the formation of zinc nanoparticles, the reaction mixture—where Pteris plant extract was exposed to 0.1 M zinc sulphate solution—was analyzed using UV-Visible spectrophotometry. A noticeable color change from dark green to a light yellowish-green was observed, which visually indicated that zinc nanoparticles had likely formed. The UV-Vis spectrum of the samples was recorded between 300 to 800 nm using a GENESYS 50 spectrophotometer. All three samples- OD culture 1, OD culture 2, and OD culture 3—showed absorption peaks at around 425 nm with absorbance values of 0.367, 0.366, and 0.366, respectively. Another minor peak was observed around 675 nm, with absorbance values ranging from 0.217 to 0.221 [Fig: 4.2]. Although zinc nanoparticles don't usually produce a very sharp surface plasmon resonance (SPR) peak like silver or gold, the broad absorption at 425 nm suggests that nanoparticles were successfully synthesized. This is likely due to the movement of electrons on the surface of the zinc particles, which is influenced by their size, shape, and the presence of bioactive compounds from the Pteris extract acting as stabilizers.



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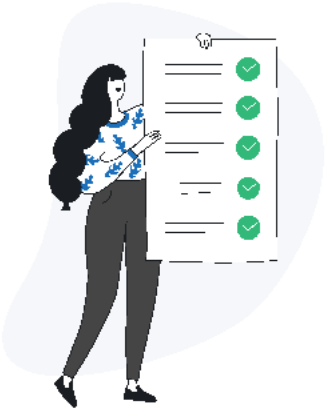
Sample OD culture 1 OD culture 2 OD culture 3
Absorbance (425) 0.367 0.366 0.366
Absorbance (675) 0.217 0.219 0.221
Table 4.1 : UV- visible spectra observed data.

Fig. 4.2 UV-Vis spectra showing SPR peak at 425 nm for all samples, confirming ZnO-NPs formation.

Our findings are in line with previous studies. For instance, researchers like Ramesh et al. (2014)(125) and Suresh et al. (2015)(126) also reported similar absorbance ranges when using plant and fungal sources for the green synthesis of zinc nanoparticles. Overall, the UV-Vis results confirm that Pteris extract effectively aided in the green synthesis of zinc nanoparticles. The consistent absorption values across samples also suggest that the nanoparticles were stable and well-dispersed, likely due to natural capping agents in the extract.



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4.2.2. DLS Analysis:

The DLS (Dynamic Light Scattering) measurement was performed to determine the particle size distribution of the green-synthesized zinc nanoparticles . The analysis revealed that the nanoparticles were fairly mono dispersed in nature, with a size distribution ranging between 20–150 nm. That's indicating effective capping and stabilization by the phytochemicals present in the Pteris extract. These findings are consistent with earlier reports on biogenic nanoparticle synthesis. For instance, Saha et al. (2010)(127) synthesized silver nanoparticles using the fungus Bipolaris nodulosa, with sizes ranging from 10–60 nm. Similarly, Jyoti et al. (2016) (128)reported the formation of silver nanoparticles in the range of 19–53 nm, while Husseiny et al. (2015)(129) synthesized silver nanoparticles using Fusarium oxysporum within a similar size distribution. The slightly higher particle size observed in the current study may be due to the intrinsic properties of zinc and the natural biomolecules from the Pteris extract acting as reducing and stabilizing agents.

4.2.3. XRD analyses of Zinc oxide Nanoparticles:

The XRD pattern of the green-synthesized ZnO nanoparticles by using Pteris extract is shown in Fig [4.3]. The diffraction data was taken in the 2θ range of 10° to 70°.

The pattern exhibits the following key features:

• A broad hump is observed between 20° and 35° (2θ), with no sharp or well-defined diffraction peaks.

• The overall intensity was get decreases by gradually with increasing 2θ.

• The absence of distinct crystalline peaks indicates that the sample lacks long-range crystallinity.

These observations suggest that the synthesized ZnO nanoparticles are predominantly amorphous or poorly crystalline in nature. The broad hump in the XRD pattern is characteristic of materials with short-range order, typical of amorphous structures or ultra-small nanocrystalline domains. (130) Such structural features are often seen in green-synthesized nanoparticles, where the use of plant extracts (in this case, Pteris extract) provides a biological reduction and stabilization environment that may hinder full crystallization. The presence of organic

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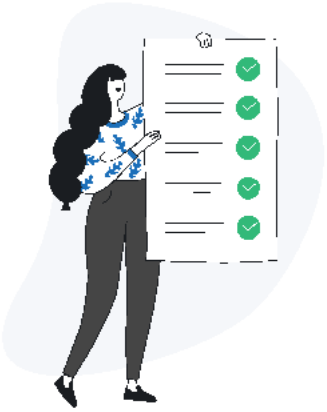
molecules such as flavonoids, polyphenols, and other phytochemicals in the extract can act as capping agents, leading to restricted crystal growth and resulting in either an amorphous matrix or extremely fine nanocrystals.

Moreover, the lack of a thermal post-treatment step (such as calcination or annealing) likely contributes to the preservation of the amorphous nature of the sample. If a more crystalline structure is desired, annealing at elevated temperatures (e.g., 400–600°C) could be employed to enhance crystal growth and improve phase purity.(131)

Overall, the XRD results confirm the formation of amorphous or nanocrystalline ZnO nanoparticles, stabilized by Pteris extract, which is consistent with typical outcomes of green synthesis methods.

Fig 4.3: Representative XRD pattern of Zinc nanoparticles.



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4.2.4. FTIR Analyses of Zinc Nanoparticles:

FTIR measurements were conducted to identify the possible interactions between zinc ions and Pteris extract during the green synthesis of zinc nanoparticles. Figure (4.4) displays the FTIR absorption spectra of the Pteris extract (Spectrum B) and the synthesized zinc nanoparticles (Spectrum A). The comparison of these spectra reveals several characteristic absorption bands which suggest the involvement of functional groups from the Pteris extract in the reduction and stabilization of zinc nanoparticles.

Both spectra exhibited a broad band around 3,420–3,440 cm^{-1}, attributed to O–H stretching vibrations of hydroxyl groups, indicating the presence of phenolic compounds and flavonoids—common secondary metabolites in Pteris species. These compounds are known to act as reducing agents in the biosynthesis of metal nanoparticles (Jin and Bai, 2002; Socrates, 2004).(132;133)

The peaks near 2,920 cm^{-1} correspond to C–H stretching vibrations, which are typically associated with aliphatic hydrocarbon chains. This suggests the presence of long-chain organic molecules such as terpenoids or fatty acids.

A significant band appeared around 1,630 1,650 cm^{-1}, representing amide I (C=O stretching) from proteins or peptides. This indicates that proteinaceous compounds in the extract may have bound to the nanoparticle surface, serving as capping agents (Barth, 2007). In addition, a band around 1,380–1,450 cm^{-1} is associated with symmetric $–COO^-$ stretching, also found in amino acids and organic acids.

Another noticeable band at approximately 1,070 cm^{-1} corresponds to C–O–C or C–O stretching, which is characteristic of alcohols, esters, and polysaccharides. These components are also abundant in plant extracts and may contribute to both reduction and stabilization processes.

Importantly, the fingerprint region between 500 and 750 cm^{-1} exhibited subtle peaks indicating bending vibrations associated with R–CH groups, possibly from poly-phenolic compounds, which are widely reported in Pteris vittata and related species (Singh et al., 2010).(134)

According to Lin et al. (2005) (135), carbonyl groups from amino acids and peptides show a high affinity for metal ions and likely played a crucial role in coordinating and stabilizing the zinc nanoparticles. Proteins and other macromolecules could form a bio-

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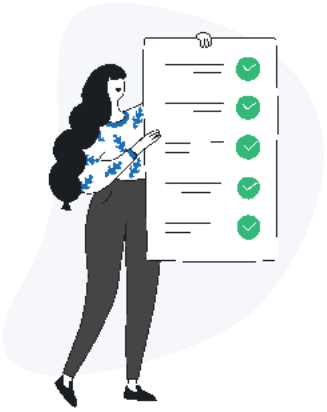
Fig 4.6: Graphical analysis of zone of inhibition by ; E. coli and Bacillus sp.
These findings are in line with other studies that also explored the green synthesis of ZnO nanoparticles. For example, a analysis by Ramesh et al. (2014) used; Aloe vera extract for ZnO nanoparticle synthesis and reported similar antimicrobial effects against both Gram-positive and Gram-negative bacteria, supporting the effectiveness of plant-mediated ZnO NPs. This simple yet eco-friendly method using Pteris extract could be a step forward in developing natural antimicrobial alternatives, especially important at a time when antibiotic resistance is a growing concern.
4.3.2. Broth micro dilution Assay Analysis of ZnO Nanoparticles Synthesized from Pteris Plant:
To determine the antimicrobial efficacy of green-synthesized zinc oxide nanoparticles (ZnO NPs) from Pteris plant extract, a broth micro dilution assay was conducted against two bacterial strains: Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive). The assay tested varying concentrations of ZnO-NPs (0.25–1.25 mM), and the percentage of bacterial growth inhibition was calculated. The results, depicted in Fig: (4.7), revealed a clear dose-dependent reduction in bacterial growth in both strains.
Among the two, S. aureus demonstrated greater sensitivity, exhibiting up to 83.2% inhibition at 1.25 mM, whereas E. coli showed a lower inhibition of 72.1% at the same concentration. These



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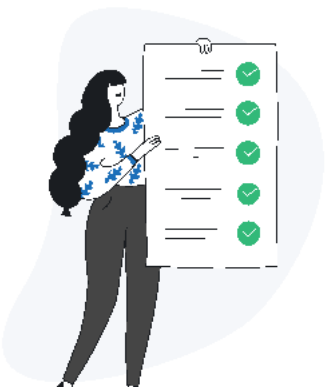
findings align with prior studies suggesting that Gram-positive bacteria, with their thick peptidoglycan wall, may have greater surface interaction with metallic nanoparticles, while Gram-negative bacteria, with an additional lipopolysaccharide-rich outer membrane, exhibit comparatively more resistance to nanoparticle penetration and action.
To further investigate the antibacterial mechanism of ZnO- NPs, membrane integrity assays were performed. These included quantification of DNA and protein leakage, which serves as a marker for membrane disruption and cell lysis.

Fig 4.7: Broth micro dilution assay; showing it's growth inhibition percentages of E. coli and S. aureus treated with ZnO-NPs.
To further investigate the antibacterial mechanism of ZnO- NPs, membrane integrity assays were performed. These included quantification of DNA and protein leakage, which serves as a marker for membrane disruption and cell lysis.
In E. coli, treatment with ZnO NPs synthesized using thermal extract (Zn-TE) resulted in a significant increase in absorbance at 260 nm (DNA) and 280 nm (protein), indicating leakage of nucleic acids and proteins into the extracellular medium. Specifically, DNA leakage increased



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Overall, the results show a clear dose-dependent antibacterial effect — higher concentrations of ZnO nanoparticles were more effective in stopping bacterial growth. By a graphical analysis Fig: (4.6), Bacillus subtilis, interestingly, appeared to be slightly more sensitive than E. coli, especially at the lower concentrations. (139)

Table 4.2: Zone of Inhibition Data

SL. No. Name of Nanoparticle E. coli (cm) Bacillus (cm) Zone of Inhibition (cm) Status (S-R)

1. ZnSo4+ Ptoris Extract.(control) 0 0 E. coli: 0 R

Bacillus: 0

2. 100% 4 4 E. coli: 3.5 S

Bacillus: 3.5

3. 50% 2.9 3 E. coli: 2.4 S

Bacillus: 2.5

4. 25% 1.2 2.2 E. coli: 0.7 R

Bacillus: 1.7

Note:

- S: Sensitive
- R: Resistance
- As zinc nanoparticles concentration increases, the zone of inhibition increases, indicating greater antibacterial effectiveness.

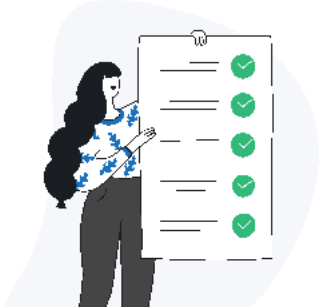

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Fig 4.6: Graphical analysis of zone of inhibition by ; E. coli and Bacillus sp.
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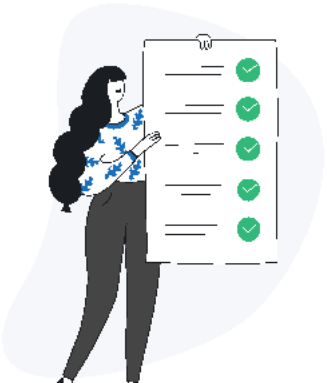

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peptidoglycan wall, may have greater surface interaction with metallic nanoparticles, while Gram-negative bacteria, with an additional lipopolysaccharide-rich outer membrane, exhibit comparatively more resistance to nanoparticle penetration and action.
To further investigate the antibacterial mechanism of ZnO- NPs, membrane integrity assays were performed. These included quantification of DNA and protein leakage, which serves as a marker for membrane disruption and cell lysis.

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In E. coli, treatment with ZnO NPs synthesized using thermal extract (Zn-TE) resulted in a significant increase in absorbance at 260 nm (DNA) and 280 nm (protein), indicating leakage of nucleic acids and proteins into the extracellular medium. Specifically, DNA leakage increased from 0.33 (control) to 0.64, while protein leakage rose from 0.27 to 0.46. These results are presented in Fig (4.8).

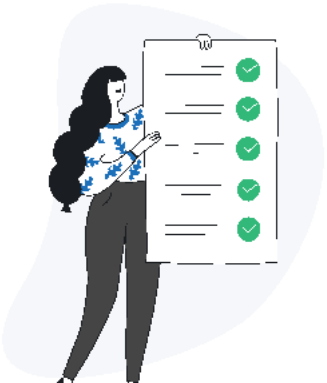

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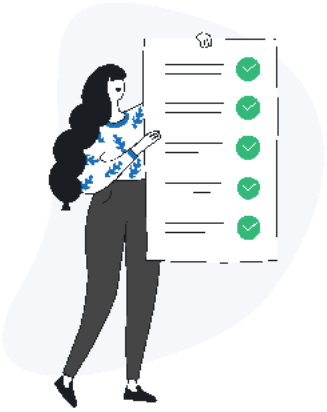
Fig 4.8: DNA (260 nm) and protein (280 nm) leakage in E. coli cells treated with ZnO-NPs
Similarly, in S. aureus, ZnO-NPs treatment caused a rise in DNA leakage from 0.28 to 0.58, and protein leakage from 0.22 to 0.42, shown in Fig (4.9). This confirms that ZnO -NPs compromise the bacterial cell membrane, ultimately leading to cell death.

Fig 4.9: DNA (260 nm) and the protein leakage (280 nm) in S. aureus cells treated by ZnO-NPs.

These outcomes are consistent with earlier reports, such as Singh et al. (2021) (140), who observed similar membrane leakage patterns in bacteria treated with neem-synthesized ZnO-NPs, and Chatterjee et al. (2019) (141), who demonstrated nanoparticle-induced leakage in clinical pathogens.



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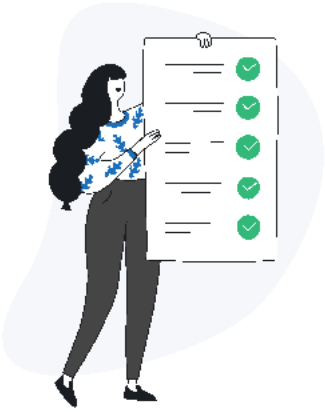
Chapter: 5
Conclusion:
This study successfully explored a green and eco-friendly method to create zinc oxide nanoparticles (ZnO-NPs) using "Pteris vittata" plant extract. The natural compounds in the plant acted as both reducing and stabilizing agents, allowing the formation of nanoparticles without the use of harmful chemicals. These all ZnO-NPs were showed a strong antibacterial activity against both Gram-positive and Gram-negative bacteria. The results from various tests like UV-Vis spectroscopy, FTIR, XRD, and DLS confirmed the successful synthesis and stability of the particles, reinforcing the idea that Pteris can be a powerful and sustainable source for nanoparticle production with potential in medical and environmental fields.
Looking forward, there's a lot of scope to build on this work. Future studies can dive deeper into understanding how these nanoparticles interact with living cells and ensure they are safe for broader use. There's also potential to fine-tune the synthesis process for better results, create practical applications like wound healing gels or antibacterial coatings, and even scale up production for industrial use. Exploring other plants or similar species might also uncover even more effective options for green nanoparticle synthesis.



No Plagiarism Found